Time Series Analysis in AFNI

Outline: 6+ Hours of Edification

- Philosophy (e.g., theory without equations)
- Sample FMRI data
- Theory underlying FMRI analyses: the **HRF**
- "Simple" or "Fixed Shape" regression analysis
  - ★ Theory and Hands-on examples
- "Deconvolution" or "Variable Shape" analysis
  - ★ Theory and Hands-on examples
- Advanced Topics (followed by brain meltdown)

Goals: Conceptual Understanding + Prepare to Try It Yourself

Data Analysis Philosophy

- **Signal** = Measurable response to stimulus
- **Noise** = Components of measurement that interfere with detection of signal
- Statistical detection theory:
  - ★ Understand relationship between stimulus & signal
  - ★ Characterize noise statistically
  - ★ Can then devise methods to distinguish noise-only measurements from signal+noise measurements, and assess the methods' reliability
  - ★ Methods and usefulness depend strongly on the assumptions
    - o Some methods are more "robust" against erroneous assumptions than others, but may be less sensitive

FMRI Philosopy: Signals and Noise

- FMRI Stimulus→Signal connection and noise statistics are both complex and poorly characterized
- Result: there is no "**best**" way to analyze FMRI time series data: there are only "**reasonable**" analysis methods
- To deal with data, must make some assumptions about the signal and noise
- Assumptions will be wrong, but must do ***something***
- Different kinds of experiments require different kinds of analyses
  - ⋆ Since signal models and questions you ask about the signal will vary
  - ⋆ It is important to understand what is going on, so you can select and evaluate "reasonable" analyses

Meta-method for creating analysis methods

- Write down a mathematical model connecting stimulus (or "activation") to signal
- Write down a statistical model for the noise
- Combine them to produce an equation for measurements given signal+noise
  - ⋆ Equation will have unknown parameters, which are to be estimated from the data
  - ⋆ N.B.: signal may have zero strength (no "activation")
- Use statistical detection theory to produce an algorithm for processing the measurements to assess signal presence and characteristics
  - ⋆ e.g., least squares fit of model parameters to data

Time Series Analysis on Voxel Data

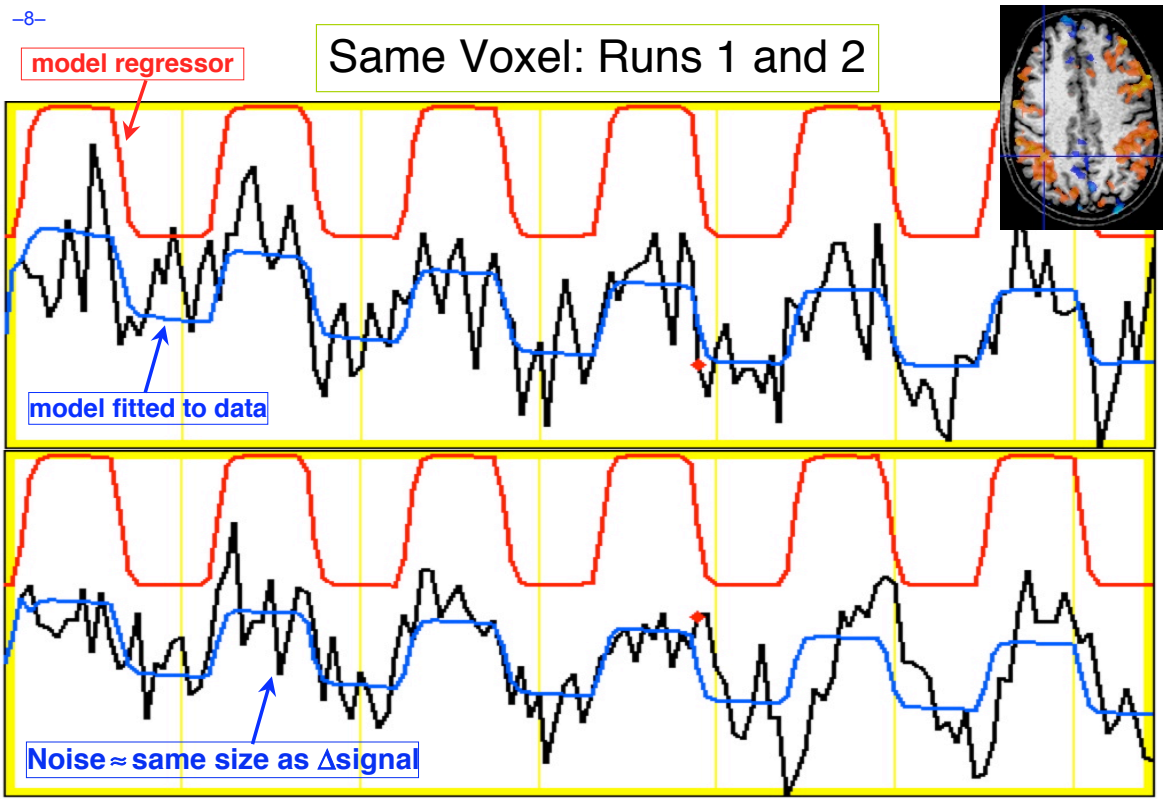
- Most common forms of FMRI analysis involve fitting an activation+BOLD model to each voxel's time series ***separately*** (AKA "univariate" analysis)
  - ⋆ Some pre-processing steps do include inter-voxel computations; e.g.,
    - o spatial smoothing to reduce noise
    - o spatial registration to correct for subject motion
- Result of model fits is a set of parameters at each voxel, estimated from that voxel's data
  - ⋆ e.g., activation amplitude (β), delay, shape
  - ⋆ "SPM" = statistical parametric map; e.g., t or F
- Further analysis steps operate on individual SPMs
  - ⋆ e.g., combining/contrasting data among subjects

Some Features of FMRI Voxel Time Series

- FMRI only measures changes due to neural "activity"
  - ⋆ Baseline level of signal in a voxel means little or nothing about neural activity
  - ⋆ Also, baseline level tends to drift around slowly (100 s time scale or so; mostly from small subject motions)
- Therefore, an FMRI experiment must have at least 2 different neural conditions ("tasks" and/or "stimuli")
  - ⋆ Then statistically test for differences in the MRI signal level between conditions
  - ⋆ Many experiments: one condition is "rest"
- Baseline is modeled separately from activation signals, and baseline model includes "rest" periods

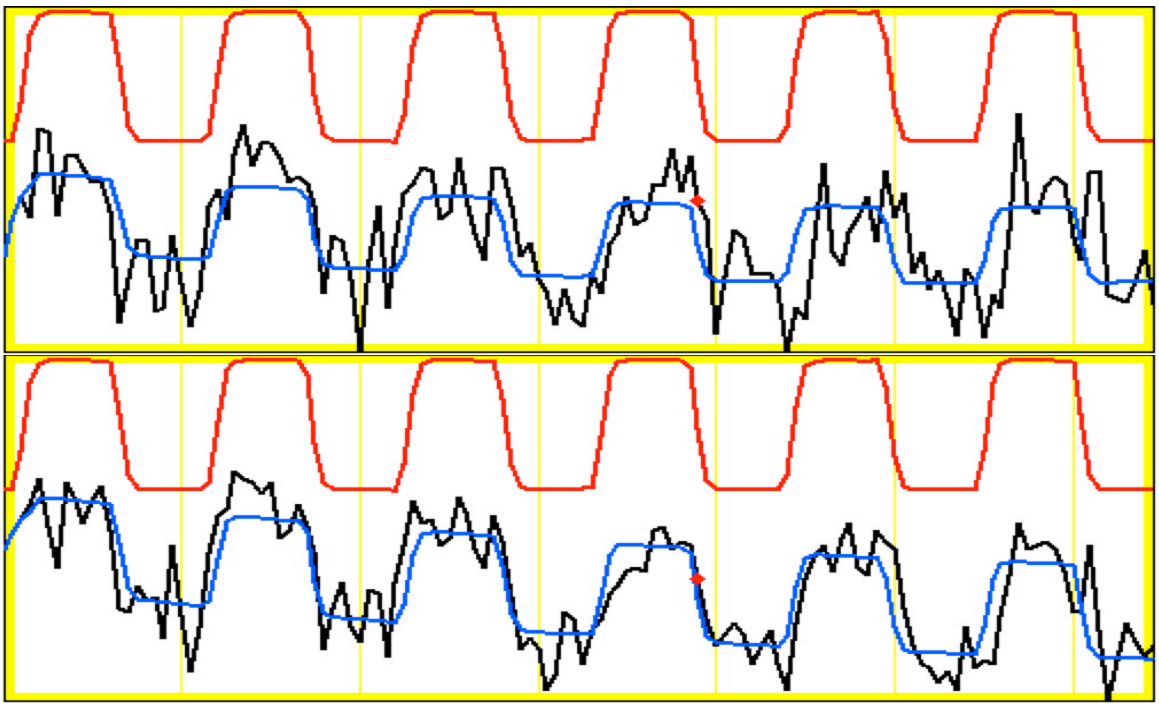
Some Sample FMRI Data Time Series

- First: Block-trial FMRI data
  - ★ "Activation" occurs over a sustained period of time (say, 10 s or longer), usually from more than one stimulation event, in rapid succession
  - ★ BOLD (hemodynamic) response accumulates from multiple close activations and is large
  - ★ BOLD response is often visible in time series
  - ★ Noise magnitude about same as BOLD response
- Next 2 slides: same brain voxel in 3 (of 9) EPI runs
  - ★ **black curve** (noisy) = data
  - ★ **red curve** (above data) = ideal model response
  - ★ **blue curve** (within data) = model fitted to data
  - ★ somatosensory task (finger being rubbed)



Block-trials: 27 s "on" / 27 s "off"; TR=2.5 s; 130 time points/run

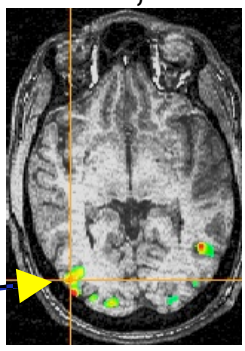
Same Voxel: Run 3 and Average of all 9



⇒ Activation amplitude and shape are variable! Why???

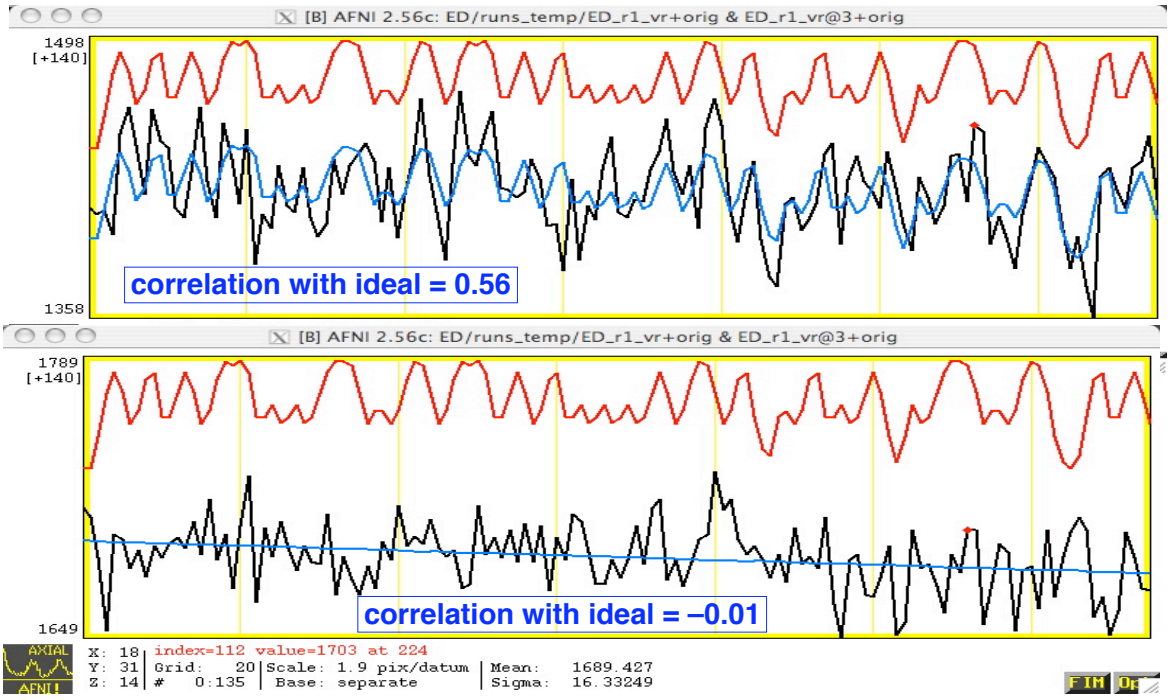
More Sample FMRI Data Time Series

- Second: Event-related FMRI
  - ★ "Activation" occurs in single relatively brief intervals
  - ★ "Events" can be randomly or regularly spaced in time
    - o If events are randomly spaced in time, signal model itself looks noise-like (to the pitiful human eye)
  - ★ BOLD response to stimulus tends to be weaker, since fewer nearby-in-time "activations" have overlapping signal changes (hemodynamic responses)
- Next slide: Visual stimulation experiment



"Active" voxel shown in next slide

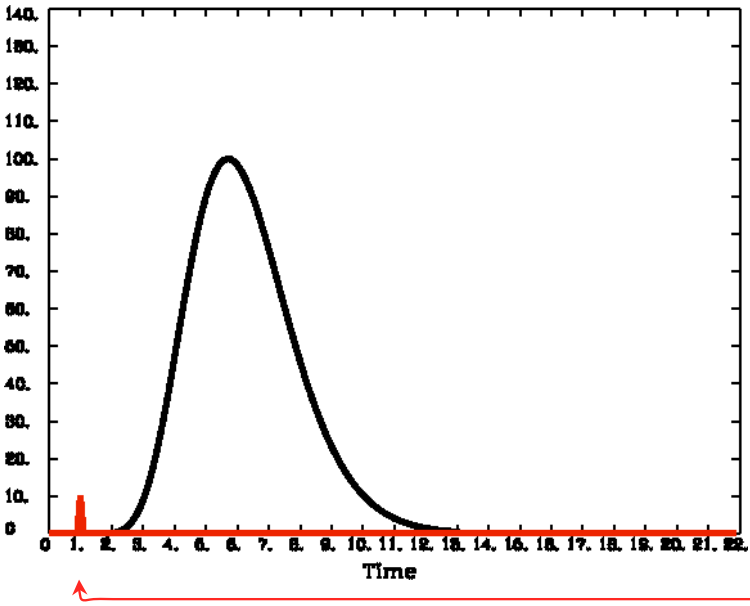
Two Voxel Time Series from Same Run



Lesson: ER-FMRI activation is not obvious via casual inspection

Hemodynamic Response Function (HRF)

- **HRF** is the idealization of measurable FMRI signal change responding to a single activation cycle (up and down) from a stimulus in a voxel



Response to brief activation (< 1 s):
- delay of 1-2 s
- rise time of 4-5 s
- fall time of 4-6 s
- model equation:

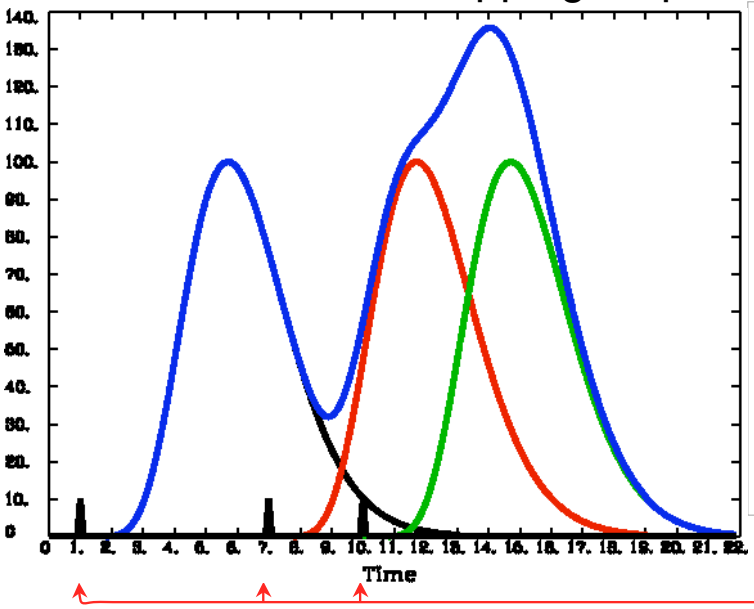
$$h(t) \propto t^{b} e^{-t/c}$$

- $h(t)$ is signal change t seconds ***after*** activation

1 Brief Activation (Event)

Linearity of HRF

- Multiple activation cycles in a voxel, closer in time than duration of HRF:
  - ⋆ Assume that overlapping responses add

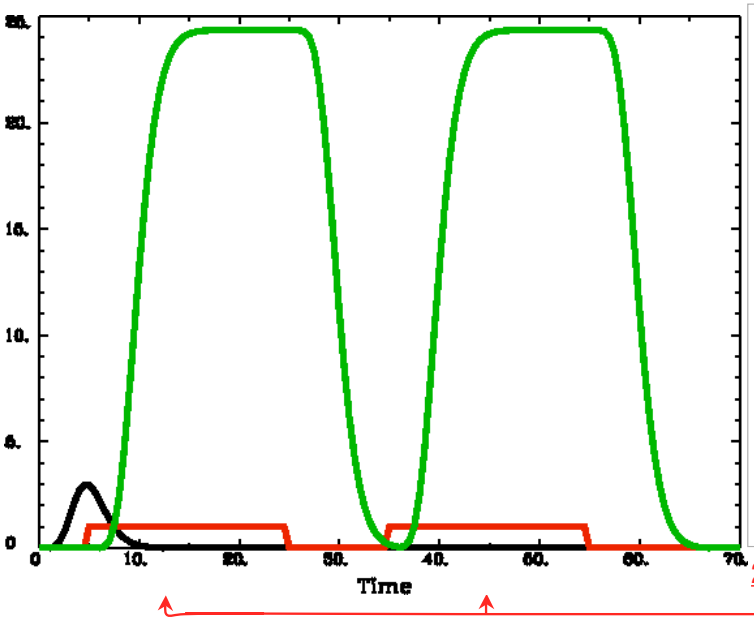


- Linearity is a pretty good assumption
- But not apparently perfect — about 90% correct
- Nevertheless, is widely taken to be true and is the basis for the "general linear model" (GLM) in FMRI analysis

3 Brief Activations

Linearity and Extended Activation

- Extended activation, as in a block-trial experiment:
  - ⋆ HRF accumulates over its duration (≈ 10 s)

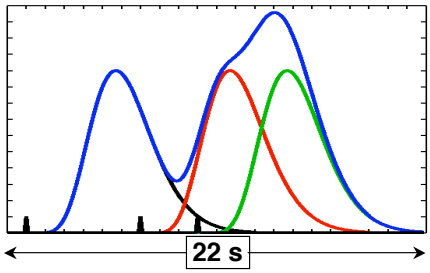


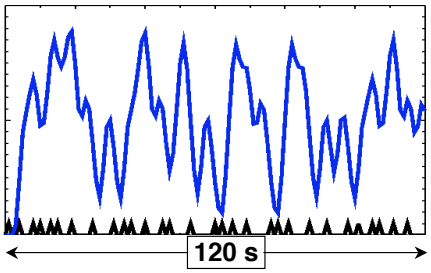
- **Black** curve = response to a single brief stimulus
- **Red** curve = activation intervals
- **Green** curve = summed up HRFs from activations
- Block-trials have larger BOLD signal changes than event-related experiments

2 Long Activations (Blocks)

Convolution Signal Model

- FMRI signal we look for in each voxel is taken to be sum of the individual trial HRFs
  - ⋆ Stimulus timing is assumed known (or measured)
  - ⋆ Resulting time series (**blue** curves) are called the ***convolution*** of the HRF with the stimulus timing
- Must also allow for baseline and baseline drifting
  - ⋆ Convolution models only the FMRI signal **changes**





- Real data starts at and returns to a nonzero, slowly drifting baseline

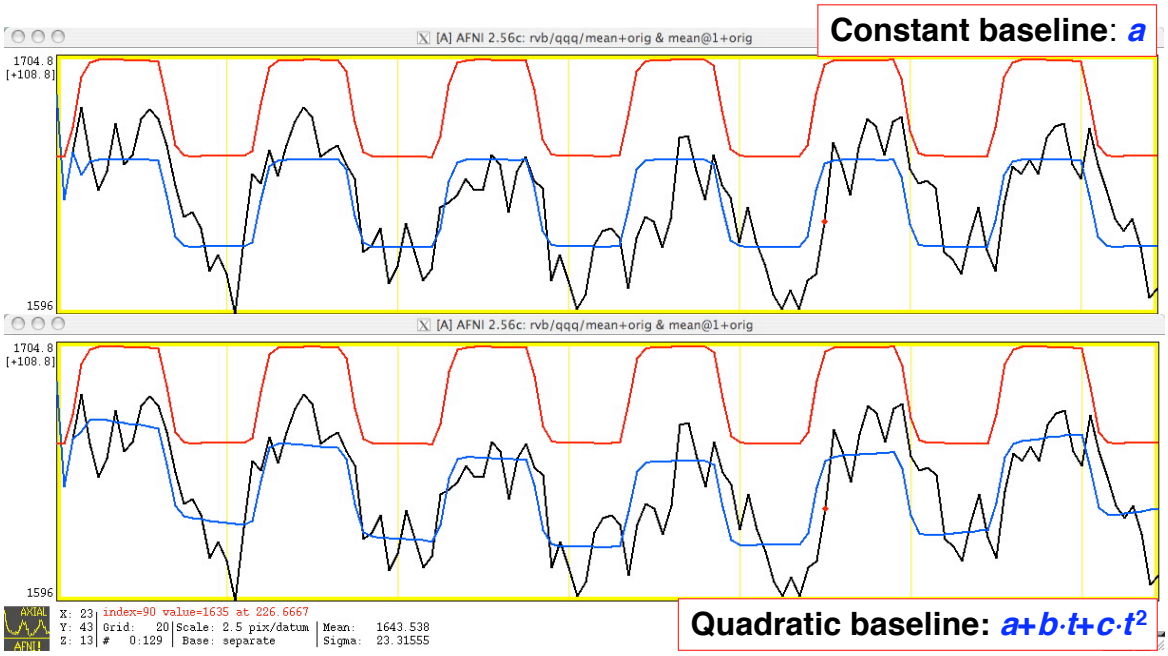
Simple Regression Models

- Assume a fixed shape $h(t)$ for the HRF
  - ⋆ e.g., $h(t) = t^{8.6} \exp(-t/0.547)$ [MS Cohen, 1997]
  - ⋆ Convolved with stimulus timing (e.g., AFNI program **`waver`**), get ideal response function $r(t)$
- Assume a form for the baseline
  - ⋆ e.g., $a + b \cdot t$ for a constant plus a linear trend
- In each voxel, fit data $Z(t)$ to a curve of the form

$$Z(t) \approx a + b \cdot t + \beta \cdot r(t)$$ ← The signal model!

  - a, b, β are unknown parameters to be calculated in each voxel
  - a,b are "nuisance" parameters
  - β is amplitude of $r(t)$ in data = "how much" BOLD

Simple Regression: Example



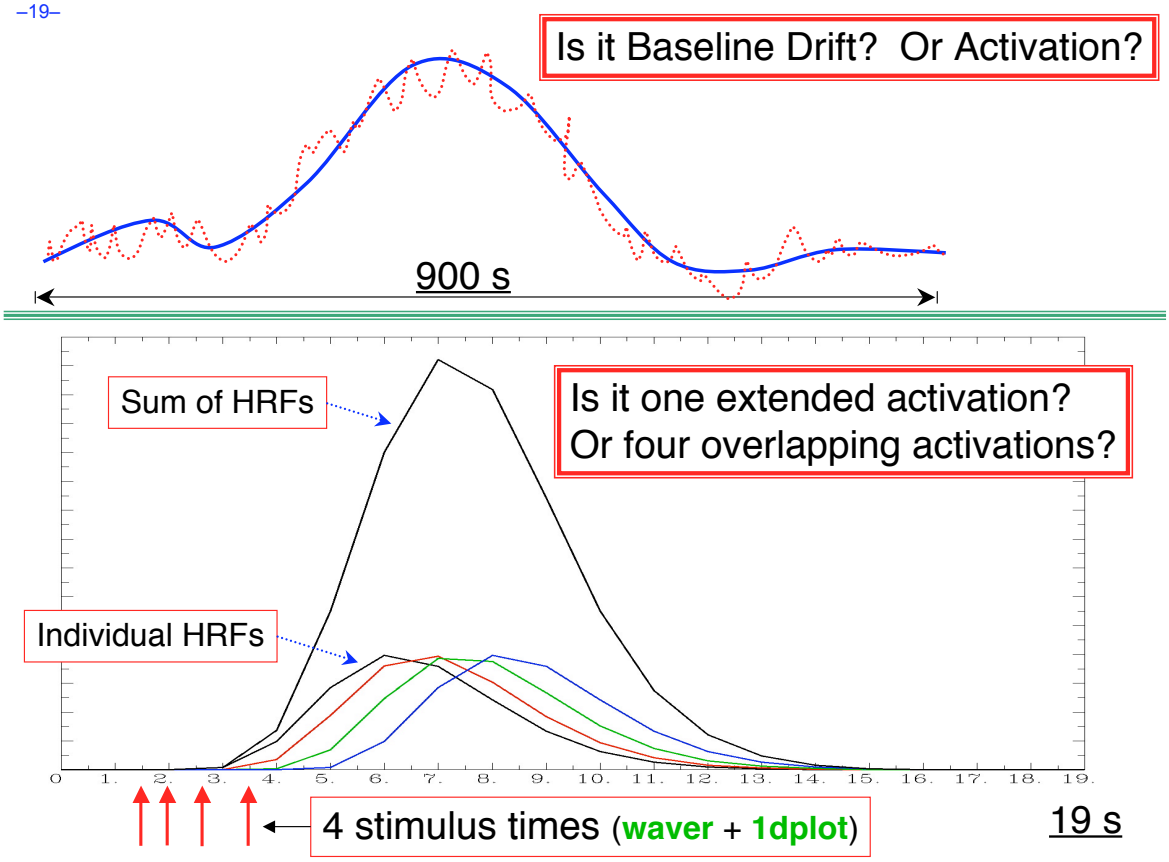
Constant baseline: ***a***

Quadratic baseline: $a+b \cdot t+c \cdot t^2$

- Necessary baseline model complexity depends on duration of **continuous** imaging — e.g., 1 parameter per ~150 seconds

Duration of Stimuli - Important Caveats

- Slow baseline drift (time scale 100 s and longer) makes doing FMRI with long duration stimuli difficult
  - Learning experiment, where the task is done continuously for ~15 minutes and the subject is scanned to find parts of the brain that adapt during this time interval
  - Pharmaceutical challenge, where the subject is given some psychoactive drug whose action plays out over 10+ minutes (e.g., cocaine, ethanol)
- Multiple very short duration stimuli that are also very close in time to each other are very hard to tell apart, since their HRFs will have 90-95% overlap
  - Binocular rivalry, where percept switches ~ 0.5 s



Multiple Stimuli = Multiple Regressors

- Usually have more than one class of stimulus or activation in an experiment
  - ★ e.g., want to see size of "**face activation**" vis-à-vis "**house activation**"; or, "**what**" vs. "**where**" activity
- Need to model each separate class of stimulus with a separate response function $r_1(t)$, $r_2(t)$, $r_3(t)$, ….
  - ★ Each $r_j(t)$ is based on the stimulus timing for activity in class number j
  - ★ Calculate a β_j amplitude = amount of $r_j(t)$ in voxel data time series $Z(t)$
  - ★ Contrast βs to see which voxels have differential activation levels under different stimulus conditions
    - o e.g., statistical test on the question $\beta_1 - \beta_2 = 0$?

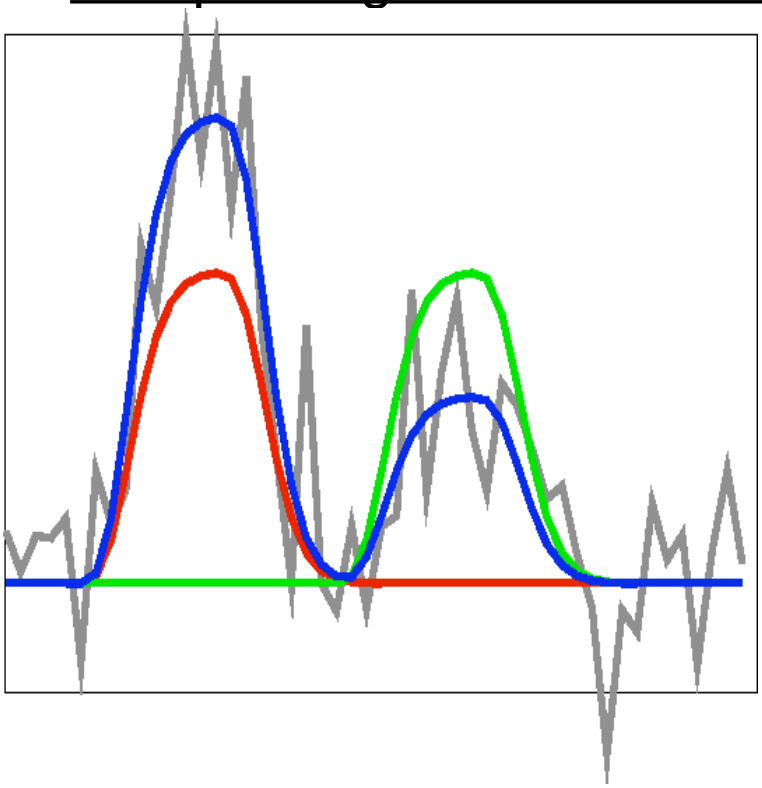
Multiple Stimuli - Important Caveat

- You do ***not*** model the baseline condition
  - e.g., “rest”, visual fixation, high-low tone discrimination, or some other simple task
- FMRI can only measure ***changes*** in MR signal levels between tasks
  - So you need some simple-ish task to serve as a reference point
- The baseline model (e.g., $a + b \cdot t$) takes care of the signal level to which the MR signal returns when the “active” tasks are turned off
  - Modeling the reference task explicitly would be redundant (or “collinear”, to anticipate a forthcoming jargon word)

Multiple Stimuli - Experiment Design

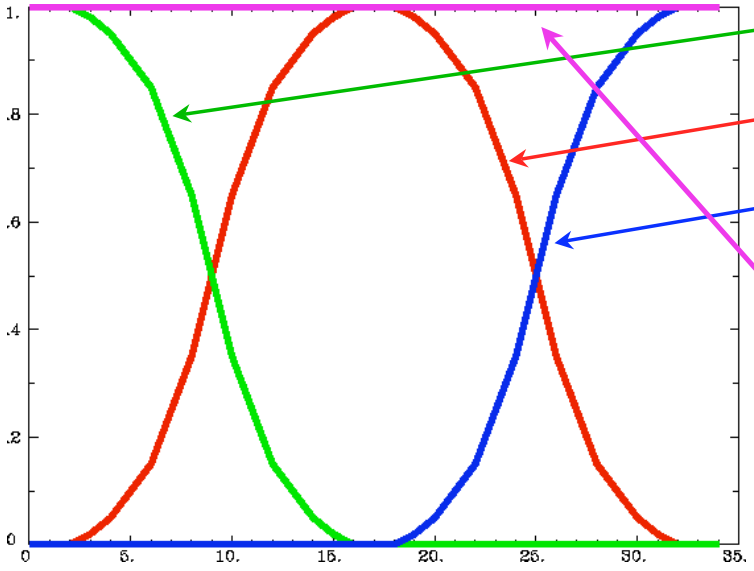
- How many distinct stimuli do you need in each class? Our rough recommendations:
  - Short event-related designs: at least 25 events in each stimulus class (spread across multiple imaging runs) — and more is better
  - Block designs: at least 5 blocks in each stimulus class — 10 would be better
- While we’re on the subject: How many subjects?
  - Several independent studies agree that 20-25 subjects in each category are needed for highly reliable results
  - This number is more than has usually been the custom in FMRI-based studies!

Multiple Regressors: Cartoon Animation



- **Red** curve = signal model for class #1
- **Green** curve = signal model for #2
- **Blue** curve = $\beta_1 \cdot$#1$+\beta_2 \cdot$#2 where β_1 and β_2 vary from 0.1 to 1.7 in the animation
- Goal of regression is to find β_1 and β_2 that make the blue curve best fit the data time series
- **Gray** curve = **1.5**·#1+**0.6**·#2+noise = simulated data

Multiple Regressors: Collinearity!!

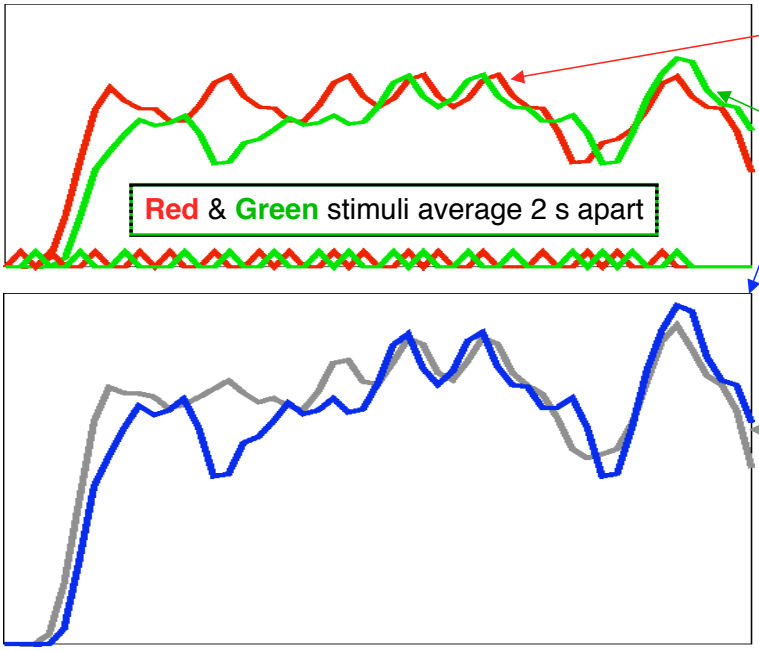


- **Green** curve = signal model for #1
- **Red** curve = signal model for class #2
- **Blue** curve = signal model for #3
- **Purple** curve = #1 + #2 + #3 which is exactly = 1
- We cannot — ***in principle or in practice*** — distinguish sum of 3 signal models from constant baseline!!

No analysis can distinguish the cases
$Z(t)$=10+ 5·#1 and
$Z(t)$= 0+15·#1+10·#2+10·#3
and an infinity of other possibilities

Collinear designs are **bad bad bad**!

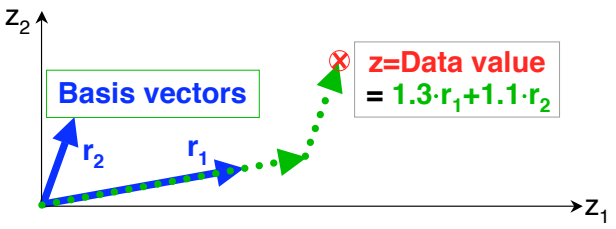
Multiple Regressors: Near Collinearity



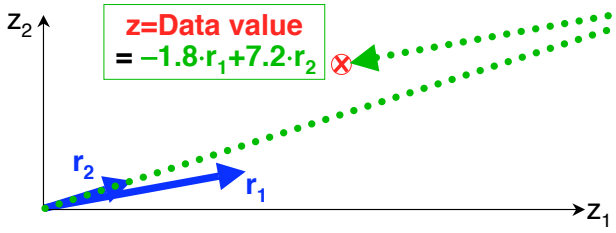
- **Red** curve = signal model for class #1
- **Green** curve = signal model for #2
- **Blue** curve = $\beta_1 \cdot$#1+(1–β_1)·#2 where β_1 varies randomly from 0.0 to 1.0 in animation
- **Gray** curve = **0.66**·#1+**0.33**·#2 = simulated data *with no noise*
- Lots of different combinations of #1 and #2 are decent fits to gray curve

Stimuli are too close in time to distinguish response #1 from #2, considering noise

The Geometry of Collinearity - 1



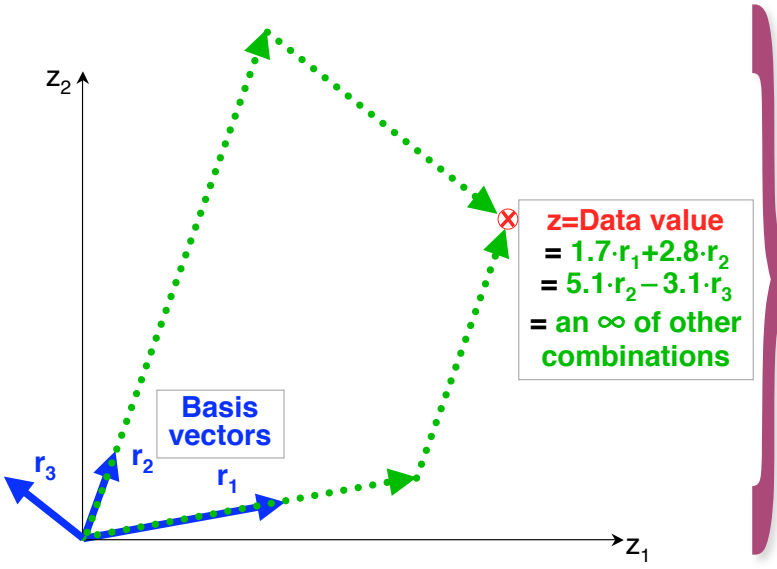
Non-collinear (well-posed)



Near-collinear (ill-posed)

- Trying to fit data as a sum of basis vectors that are nearly parallel doesn't work well: solutions can be huge
- Exactly parallel basis vectors would be impossible:
  - Determinant of matrix to invert would be zero

The Geometry of Collinearity - 2



Multi-collinear
= more than one solution fits the data
= over-determined

- Trying to fit data with too many regressors (basis vectors) doesn't work: no unique solution

Equations: Notation

- Will generally follow notation of Doug Ward's manual for the AFNI program **3dDeconvolve**
- Time: continuous in reality, but in steps in the data
  - ★ Functions of continuous time are written like $f(t)$
  - ★ Functions of discrete time expressed like $f(\underbrace{n \cdot TR}_{=t_n})$ where n=0,1,2,… and TR=time step
  - ★ Usually use subscript notion f_n as shorthand
  - ★ Collection of numbers assembled in a column is a ***vector*** and is printed in boldface:

$$\left\{\begin{matrix}\text{vector of} \\ \text{length } N\end{matrix}\right\} = \begin{bmatrix} f_0 \\ f_1 \\ f_2 \\ \vdots \\ f_{N-1} \end{bmatrix} = \mathbf{f} \qquad \begin{bmatrix} A_{00} & A_{01} & \cdots & A_{0,N-1} \\ A_{10} & A_{11} & \cdots & A_{1,N-1} \\ \vdots & \vdots & \ddots & \vdots \\ A_{M-1,0} & A_{M-1,1} & \cdots & A_{M-1,N-1} \end{bmatrix} = \mathbf{A} = \{M \times N \text{ matrix}\}$$

Equations: Single Response Function

- In each voxel, fit data Z_n to a curve of the form

  $Z_n \approx a + b \cdot t_n + \beta \cdot r_n$ for $n=0,1,\ldots,N-1$ (N=# time pts)

  - a, b, β are unknown parameters to be calculated in each voxel
  - a,b are "nuisance" baseline parameters
  - β is amplitude of $r(t)$ in data = "how much" BOLD
  - Baseline model might be more complicated for long (> 150 s) continuous imaging runs:
    - $150 < T < 300$ s: $a+b \cdot t+c \cdot t^2$
    - Longer: $a+b \cdot t+c \cdot t^2 + \lceil T/150 \rceil$ low frequency components
    - Usually, also include as extra baseline components the estimated subject head movement time series, in order to remove residual contamination from such artifacts (will see example of this later)

≈1 param per 150 s

Equations: Multiple Response Functions

- In each voxel, fit data Z_n to a curve of the form

$$Z_n \approx [\text{baseline}]_n + \beta_1 \cdot r_n^{(1)} + \beta_2 \cdot r_n^{(2)} + \beta_3 \cdot r_n^{(3)} + \cdots$$

  - β_j is amplitude in data of $r_n^{(j)}=r_j(t_n)$; i.e., "how much" of j^{th} response function in in the data time series
  - In simple regression, each $r_j(t)$ is derived directly from stimulus timing ***and*** user-chosen HRF model
    - In terms of stimulus times: $r_n^{(j)} = \sum_{k=1}^{K_j} h(t_n - \tau_k^{(j)})$
    - If stimulus occurs on the imaging TR time-grid, stimulus can be represented as a 0-1 time series: $\left[s_0^{(j)} \quad s_1^{(j)} \quad s_2^{(j)} \quad s_3^{(j)} \quad \cdots \right]$ where $s_k^{(j)}=1$ if stimulus #j is on at time $t=k \cdot \text{TR}$, and $s_k^{(j)}=0$ if #j is off at that time:

$$r_n^{(j)} = h_0 s_n^{(j)} + h_1 s_{n-1}^{(j)} + h_2 s_{n-2}^{(j)} + h_3 s_{n-3}^{(j)} + \cdots = \sum_{q=0}^{p} h_q s_{n-q}^{(j)}$$

Equations: Matrix-Vector Form

- Express ***known*** data vector as a sum of ***known*** columns with ***unknown*** coefficents:

$$\begin{bmatrix} z_0 \\ z_1 \\ z_2 \\ \vdots \\ z_{N-1} \end{bmatrix} \approx \begin{bmatrix} 1 \\ 1 \\ 1 \\ \vdots \\ 1 \end{bmatrix} \cdot a + \begin{bmatrix} 0 \\ 1 \\ 2 \\ \vdots \\ N-1 \end{bmatrix} \cdot b + \begin{bmatrix} r_0^{(1)} \\ r_1^{(1)} \\ r_2^{(1)} \\ \vdots \\ r_{N-1}^{(1)} \end{bmatrix} \cdot \beta_1 + \begin{bmatrix} r_0^{(2)} \\ r_1^{(2)} \\ r_2^{(2)} \\ \vdots \\ r_{N-1}^{(2)} \end{bmatrix} \cdot \beta_2 + \cdots$$

- Const baseline
- Linear trend

'≈' means "least squares"

or

$$\begin{bmatrix} z_0 \\ z_1 \\ z_2 \\ \vdots \\ z_{N-1} \end{bmatrix} \approx \begin{bmatrix} 1 & 0 & r_0^{(1)} & r_0^{(1)} & \cdots \\ 1 & 1 & r_1^{(1)} & r_1^{(1)} & \cdots \\ 1 & 2 & r_2^{(1)} & r_2^{(1)} & \cdots \\ \vdots & \vdots & \vdots & \vdots & \ddots \\ 1 & N-1 & r_{N-1}^{(1)} & r_{N-1}^{(2)} & \cdots \end{bmatrix} \begin{bmatrix} a \\ b \\ \beta_1 \\ \beta_2 \\ \vdots \end{bmatrix}$$

or

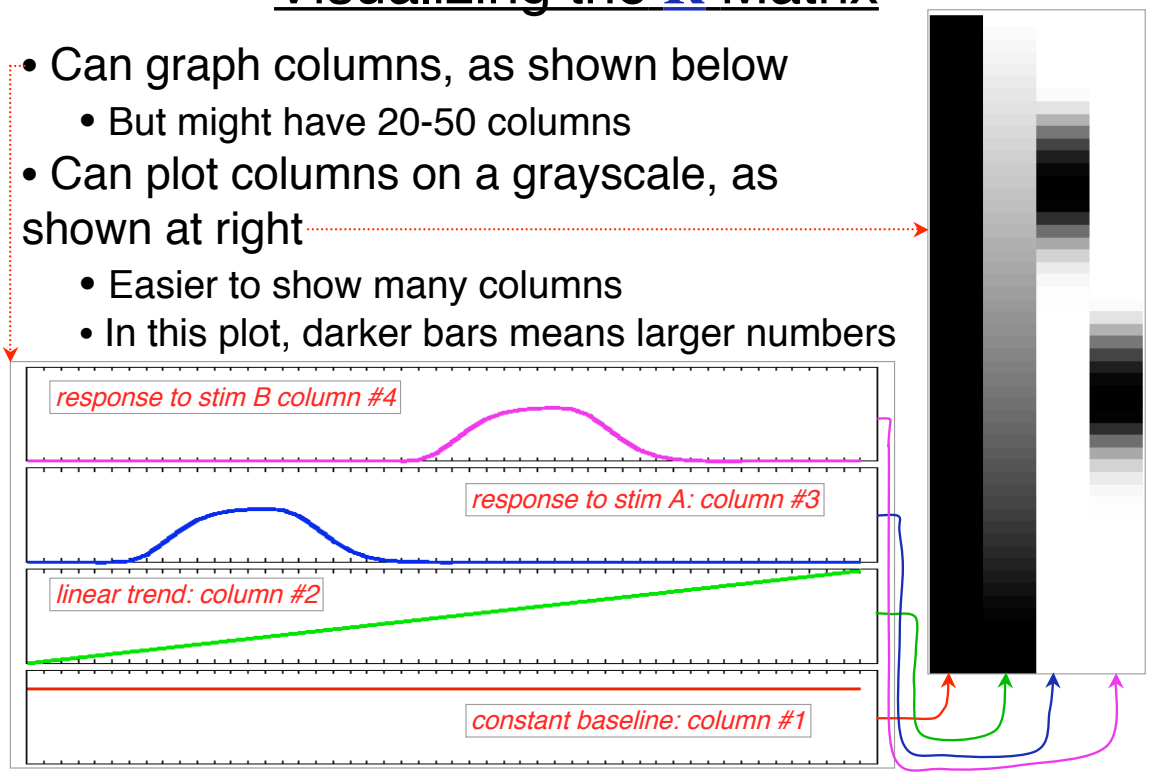
$$\underbrace{\mathbf{z}}_{\text{vector of data}} \approx \underbrace{\mathbf{R}}_{\text{matrix of columns}} \underbrace{\boldsymbol{\beta}}_{\text{vector of coeff}}$$

the "design" matrix

z depends on the voxel; **R** doesn't

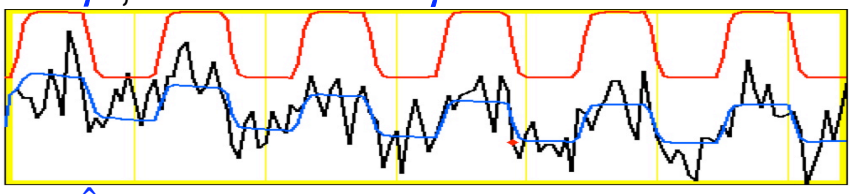
Visualizing the R Matrix

- Can graph columns, as shown below
  - But might have 20-50 columns
- Can plot columns on a grayscale, as shown at right
  - Easier to show many columns
  - In this plot, darker bars means larger numbers



Solving $\mathbf{z} \approx \mathbf{R}\beta$ for β

- Number of equations = number of time points
  - ⋆ 100s per run, but perhaps 1000s per subject
- Number of unknowns usually in range 5–50
- Least squares solution: $\hat{\beta} = \left[\mathbf{R}^T\mathbf{R}\right]^{-1}\mathbf{R}^T\mathbf{z}$
  - ⋆ $\hat{\beta}$ denotes an *estimate* of the true (unknown) β
  - ⋆ From $\hat{\beta}$, calculate $\hat{\mathbf{z}} = \mathbf{R}\hat{\beta}$ as the *fitted model*



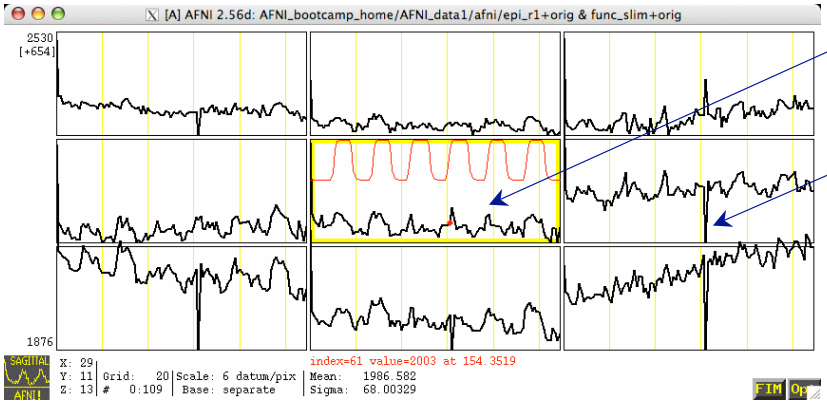
    - o $\mathbf{z} - \hat{\mathbf{z}}$ is the ***residual time series*** = noise (we hope)
- Collinearity: when matrix $\mathbf{R}^T\mathbf{R}$ can't be inverted
  - ⋆ Near collinearity: when inverse exists but is huge

Simple Regression: Recapitulation

- Choose HRF model $h(t)$ [AKA *fixed-model regression*]
- Build model responses $r_n(t)$ to each stimulus class
  - ⋆ Using $h(t)$ and the stimulus timing
- Choose baseline model time series
  - ⋆ Constant + linear + quadratic + movement?
- Assemble model and baseline time series into the columns of the $\mathbf{R}$ matrix
- For each voxel time series $\mathbf{z}$, solve $\mathbf{z} \approx \mathbf{R}\beta$ for $\hat{\beta}$
- **Individual subject maps**: Test the coefficients in $\hat{\beta}$ that you care about for statistical significance
- **Group maps**: Transform the coefficients in $\hat{\beta}$ that you care about to Talairach space, and perform statistics on these $\hat{\beta}$ values

Sample Data Analysis: Simple Regression

- Enough theory (for now: more to come later!)
- To look at the data: type `cd AFNI_data1/afni` ; then `afni`
- `Switch Underlay` to dataset `epi_r1`
  - ★ Then Sagittal `Image` and `Graph`
  - ★ `FIM→Pick Ideal` ; then click `afni/ideal_r1.1D` ; then `Set`
  - ★ Right-click in image, `Jump to (ijk)`, then `29 11 13`, then `Set`



- Data clearly has activity in sync with reference
- Data also has a big spike, which is very annoying
  - Subject head movement!

Preparing Data for Analysis

- Six preparatory steps are common:
  - ★ Image registration (realignment): program `3dvolreg`
  - ★ Image smoothing: program `3dmerge`
  - ★ Image masking: program `3dClipLevel` or `3dAutomask`
  - ★ Conversion to percentile: programs `3dTstat` and `3dcalc`
  - ★ Censoring out time points that are bad: program `3dToutcount` or `3dTqual`
  - ★ Catenating multiple imaging runs into 1 big dataset: program `3dTcat`

- Not all steps are necessary or desirable in any given case
- In this first example, will only do registration, since the data obviously needs this correction

Data Analysis Script

- In file **epi_r1_decon**:

```
waver -GAM                                  \
      -input epi_r1_stim.1D                 \
      -TR 2.5                               \
      > epi_r1_ideal.1D

3dvolreg -base 2                            \
         -prefix epi_r1_reg                 \
         -1Dfile epi_r1_mot.1D              \
         -verb                              \
         epi_r1+orig

3dDeconvolve                                \
    -input epi_r1_reg+orig                  \
    -nfirst     2                           \
    -num_stimts 1                           \
    -stim_file  1 epi_r1_ideal.1D           \
    -stim_label 1 AllStim                   \
    -tout                                   \
    -bucket epi_r1_func                     \
    -fitts  epi_r1_fitts
```

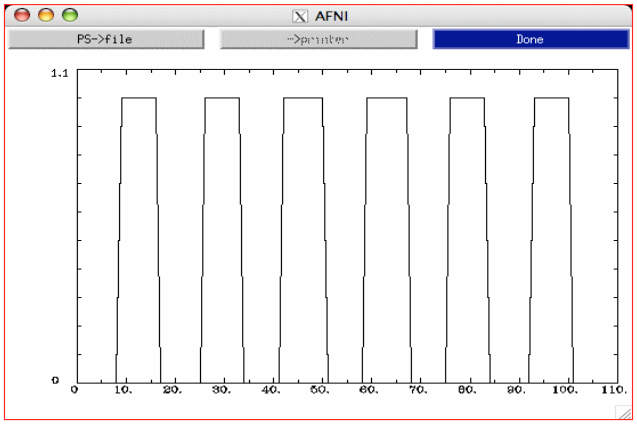
- **waver** creates model time series from input stimulus timing in file **epi_r1_stim.1D**
  - Plot a 1D file to screen with **1dplot epi_r1_ideal.1D**
- **3dvolreg** (3D image registration) will be covered in a later presentation
- **3dDeconvolve** = regression code
  - Name of input dataset
  - Index of first sub-brick to process
  - Number of input model time series
  - Name of first input model time series file
  - Name for results in AFNI menus
  - Indicates to output *t*-statistic for β weights
  - Name of output "bucket" dataset (statistics)
  - Name of output model fit dataset

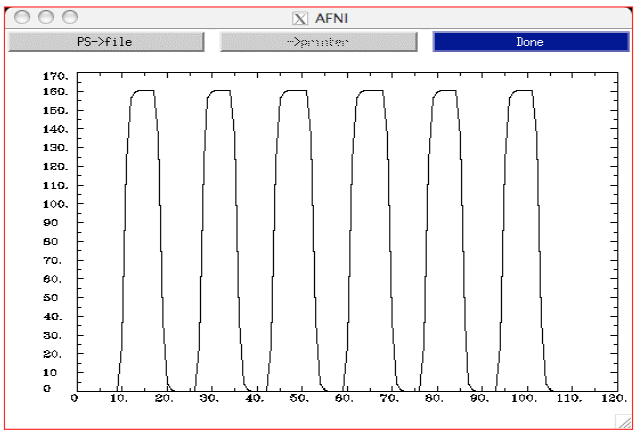
Contents of.1D files

epi_r1_stim.1D **epi_r1_ideal.1D**

epi_r1_stim.1D	epi_r1_ideal.1D
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
0	0
1	0
1	24.4876
1	122.869
1	156.166
1	160.258
1	160.547
1	160.547
1	160.547
0	160.547
0	136.059
0	37.6781
0	4.38121
0	0.288748
0	0
0	0
...	...

- 1 line per time point
- TR=2.5 s
- 0=stim OFF
- 1=stim ON
- Note that "ideal" is delayed from stimulus
- Graphs at right created with **1dplot**





To Run Script and View Results

- type **source epi_r1_decon** ; then wait for programs to run
- type **afni** to view what we've got
  - ★ **Switch Underlay** to epi_r1_reg (output from **3dvolreg**)
  - ★ **Switch Overlay** to epi_r1_func (output from **3dDeconvolve**)
  - ★ **Sagittal Image** and **Graph** viewers
  - ★ **FIM→Ignore→2** to have graph viewer not plot 1st 2 time pts
  - ★ **FIM→Pick Ideal** ; pick **epi_r1_ideal.1D** (output from **waver**)
- Define Overlay to set up functional coloring
  - **Olay→Allstim[0] Coef** (sets coloring to be from model fit β)
  - **Thr→Allstim[0] t-s** (sets threshold to be model fit *t*-statistic)
  - **See Overlay** (otherwise won't see the function!)
  - Play with threshold slider to get a meaningful activation map (e.g., *t*=4 is a decent threshold) — more on thresholds later

Textual Output of the `epi_r1_decon` script

- 3dvolreg output

```
++ Program 3dvolreg: AFNI version=AFNI_2005_12_30_0934 [32-bit]
++ Authored by: RW Cox
++ Reading input dataset ./epi_r1+orig.BRIK
++ Edging: x=3 y=3 z=1
++ Initializing alignment base
++ Starting final pass on 110 sub-bricks: 0..1..2..3.. *** ..106..107..108..109..
++ CPU time for realignment=8.82 s  [=0.0802 s/sub-brick]
++ Min : roll=-0.086  pitch=-0.995  yaw=-0.325  dS=-0.310  dL=-0.010  dP=-0.680
++ Mean: roll=-0.019  pitch=-0.020  yaw=-0.182  dS=+0.106  dL=+0.085  dP=-0.314
++ Max : roll=+0.107  pitch=+0.090  yaw=+0.000  dS=+0.172  dL=+0.204  dP=+0.079
++ Wrote dataset to disk in ./epi_r1_reg+orig.BRIK
```

- 3dDeconvolve output

```
++ Program 3dDeconvolve: AFNI version=AFNI_2005_12_30_0934 [32-bit]
++ Authored by: B. Douglas Ward, et al.
++ (108x3) Matrix condition [X]:  2.43095
++ Matrix inverse average error = 1.3332e-14
++ Matrix setup time = 0.00 s
++ Calculations starting; elapsed time=0.502
++ voxel loop:0123456789.0123456789.0123456789.0123456789.0123456789.
++ Calculations finished; elapsed time=3.114
++ Wrote bucket dataset into ./epi_r1_func+orig.BRIK
++ Wrote 3D+time dataset into ./epi_r1_fitts+orig.BRIK
++ #Flops=4.18043e+08  Average Dot Product=4.56798
```

Quality Control: Good values

Progress meter

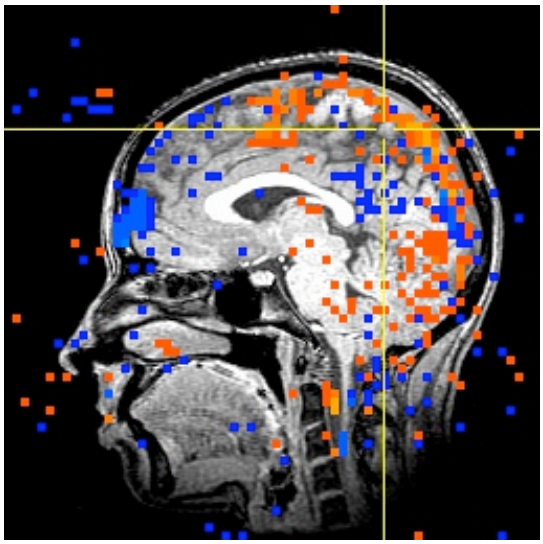
Output indicators

- If a program crashes, we'll need to see this textual output (at least)!

More Viewing the Results

- Graph viewer: **Opt→Tran 1D→Dataset #N** to plot the model fit dataset output by **3dDeconvolve**
  - Will open the control panel for the **Dataset #N** plugin
  - Click first **Input** on ; then choose **Dataset epi_r1_fitts+orig**
  - Also choose **Color dk-blue** to get a pleasing plot
  - Then click on **Set+Close** (to close the plugin panel)
  - Should now see fitted time series in the graph viewer instead of data time series
  - Graph viewer: click **Opt→Double Plot→Overlay** on to make the fitted time series appear as an overlay curve
  - This tool lets you visualize the quality of the data fit
- Can also now overlay function on MP-RAGE anatomical by using **Switch Underlay** to **anat+orig** dataset
  - Probably won't want to graph the **anat+orig** dataset!

Stimulus Correlated Movement?



• **3dvolreg** saved the motion parameters estimates into file **epi_r1_mot.1D**
• For fun: **1dplot epi_r1_mot.1D**

• Extensive "activation" (i.e., correlation of data time series with model time series) along the top of the brain is an indicator of stimulus correlated motion artifact
• Can remain even after registration, due to errors in registration, magnetic field inhomogeneities, etc.
• Can be partially removed by using the estimated movement history (from **3dvolreg**) as additional baseline model functions

Removing Residual Motion Artifacts

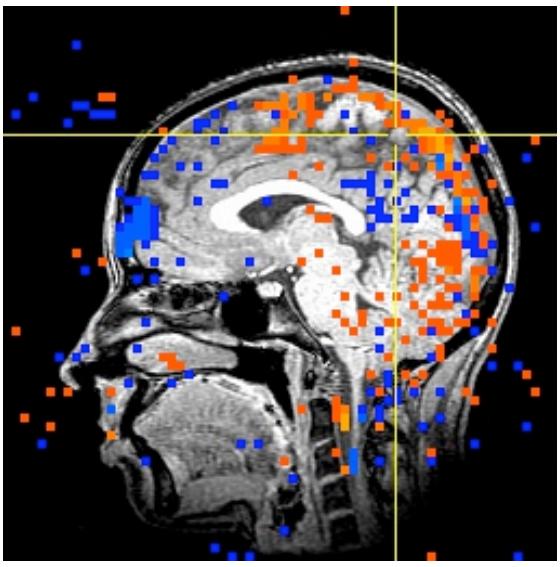
- Last part of script **epi_r1_decon**:

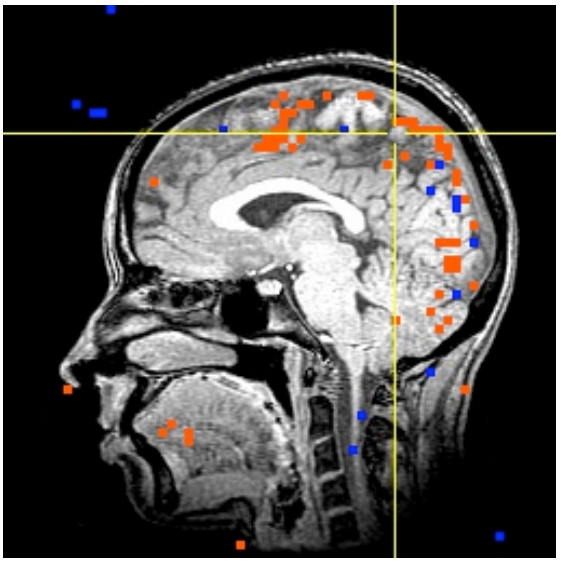
```
3dDeconvolve                                \
    -input epi_r1_reg+orig                  \
    -nfirst     2                           \
    -num_stimts 7                           \
    -stim_file  1 epi_r1_ideal.1D           \
    -stim_label 1 AllStim                   \
    -stim_file  2 epi_r1_mot.1D'[0]'        \
    -stim_base  2                           \
    -stim_file  3 epi_r1_mot.1D'[1]'        \
    -stim_base  3                           \
    -stim_file  4 epi_r1_mot.1D'[2]'        \
    -stim_base  4                           \
    -stim_file  5 epi_r1_mot.1D'[3]'        \
    -stim_base  5                           \
    -stim_file  6 epi_r1_mot.1D'[4]'        \
    -stim_base  6                           \
    -stim_file  7 epi_r1_mot.1D'[5]'        \
    -stim_base  7                           \
    -tout                                   \
    -bucket epi_r1_func_mot                 \
    -fitts  epi_r1_fitts_mot
```

These new lines add 6 regressors to the model and assign them to the baseline (**-stim_base** option)

Output files: take a moment to look at results

Some Results: Before and After





Before: movement parameters *are not* in baseline model

After: movement parameters *are* in baseline model

t-statistic threshold set to (uncorrected) *p*-value of 10^{-4} in both images

Setting the Threshold: Principles

- Bad things:
  - False positives — activations reported that aren't really there ≡ Type I error
  - False negatives — non-activations reported where there should be true activations found ≡ Type II error
- Usual approach in statistical testing is to control the probability of a type I error
- In FMRI, we are making many statistical tests: one per voxel (≈20,000+) — the result of which is an "activation map":
  - Voxels are colorized if they are survive the thresholding process

Important Aside

Setting the Threshold: Bonferroni

- If we set the threshold so there is a 1% chance that any given voxel is declared "active" even if its data is pure noise (FMRI jargon: "uncorrected" p-value is 0.01):
  - And assume each voxel's noise is independent of its neighbors (not really true)
  - With 30,000 voxels to threshold, would expect to get 300 false positives — this may be as many as the true activations! Situation: **Not so good**.
- Bonferroni solution: set threshold (e.g., on *t*-statistic) so high that uncorrected p-value is 0.05/20000=2.5e-6
  - Then have only a 5% chance that even a single false positive voxel will be reported
  - Objection: will likely lose weak areas of activation

Important Aside

Setting the Threshold: Spatial Clustering

- Cluster-based detection lets us lower the statistical threshold and still control the false positive rate
- **Two** thresholds:
  - **First**: a per-voxel threshold that is somewhat low (so by itself leads to a lot of false positives, scattered around)
  - **Second**: form clusters of spatially contiguous (neighboring) voxels that survive the first threshold, and keep only those clusters above a volume threshold — e.g., we don't keep isolated "active" voxels
- Usually: choose volume threshold, then calculate voxel-wise statistic threshold to get the overall "corrected" p-value you want (typically, corrected p=0.05)
  - No easy formulas for this, so must use simulation: AFNI program `AlphaSim`

Important Aside

AlphaSim: Clustering Thresholds

- Simulated for brain mask of 18,465 voxels
- Look for smallest cluster with corrected p < 0.05

Uncorrected p-value (per voxel)	Cluster Size / Corrected p (uncorrelated)	Cluster Size / Corrected p (correlated 5 mm)
0.0002	2 / 0.001	3 / 0.004
0.0004	2 / 0.008	4 / 0.012
0.0007	2 / 0.026	3 / 0.031
0.0010	3 / 0.001	4 / 0.007
0.0020	3 / 0.003	4 / 0.032
0.0030	3 / 0.008	5 / 0.013
0.0040	3 / 0.018	5 / 0.029
0.0050	3 / 0.030	6 / 0.012
0.0060	4 / 0.003	6 / 0.023
0.0070	4 / 0.004	6 / 0.036
0.0080	4 / 0.006	7 / 0.016
0.0090	4 / 0.010	7 / 0.027
0.0100	4 / 0.015	7 / 0.042

Corresponds to sample data

Can make activation maps for display with cluster editing using `3dmerge` program or in AFNI GUI (new: Sep 2006)

Important Aside

Multiple Stimulus Classes

- The experiment analyzed here in fact is more complicated
  - ★ There are 4 related visual stimulus types
  - ★ One goal is to find areas that are differentially activated between these different types of stimuli
  - ★ We have 4 imaging runs, 108 useful time points each (skipping first 2 in each run) that we will analyze together
    - o Already registered and put together into dataset **rall_vr+orig**
  - ★ Stimulus timing files are in subdirectory **stim_files/**
  - ★ Script file **waver_ht2** will create HRF models for regression:

```
cd stim_files
waver -dt 2.5 -GAM -input scan1to4a.1D > scan1to4a_hrf.1D
waver -dt 2.5 -GAM -input scan1to4t.1D > scan1to4t_hrf.1D
waver -dt 2.5 -GAM -input scan1to4h.1D > scan1to4h_hrf.1D
waver -dt 2.5 -GAM -input scan1to4l.1D > scan1to4l_hrf.1D
cd ..
```

  - ★ Type **source waver_ht2** to run this script
    - o Might also use **1dplot** to check if input .1D files are reasonable

Regression with Multiple Model Files

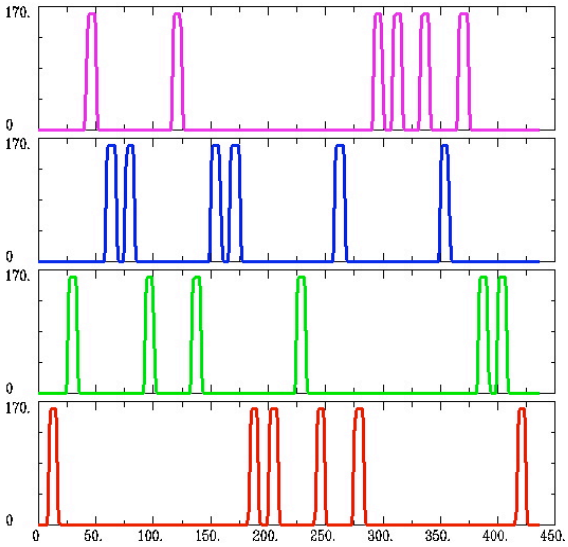
- Script file **decon_ht2** does the job:

```
3dDeconvolve -xout -input rall_vr+orig                                  \
    -num_stimts 4                                                       \
    -stim_file 1 stim_files/scan1to4a_hrf.1D  -stim_label 1 Actions     \
    -stim_file 2 stim_files/scan1to4t_hrf.1D  -stim_label 2 Tool        \
    -stim_file 3 stim_files/scan1to4h_hrf.1D  -stim_label 3 HighC       \
    -stim_file 4 stim_files/scan1to4l_hrf.1D  -stim_label 4 LowC        \
    -concat contrasts/runs.1D                                           \
    -glt 1 contrasts/contr_AvsT.txt   -glt_label 1 AvsT                \
    -glt 1 contrasts/contr_HvsL.txt   -glt_label 2 HvsL                \
    -glt 1 contrasts/contr_ATvsHL.txt -glt_label 3 ATvsHL              \
    -full_first -fout -tout                                             \
    -bucket func_ht2
```

- Run this script by typing **source decon_ht2** (takes a few minutes)

• Stim #1 = visual presentation of active movements
• Stim #2 = visual presentation of simple (tool-like) movements
• Stims #3 and #4 = high and low contrast gratings

Regressors for This Script



via **1dgrayplot** or **-xjpeg** option

via **1dplot** on **-stim_file** inputs

Extra Features of 3dDeconvolve - 1

`-concat contrasts/runs.1D` = file that indicates where new imaging runs start

```
0
108
216
324
```

`-full_first` = put **full model** statistic first in output file, not last

`-fout -tout` = output both *F*- and *t*-statistics

- The full model statistic is an *F*-statistic that shows how well the sum of all 4 input model time series fits voxel time series data
- The individual models also will get individual *F*- and *t*-statistics indicating the significance of their individual contributions to the time series fit
  - ★ i.e., F_{Actions} tells if model (**Actions+HighC+LowC+Tools+baseline**) explains more of the data variability than model (**HighC+LowC+Tools+baseline**) — with **Actions** omitted

Extra Features of 3dDeconvolve - 2

```
-glt 1 contrasts/contr_AvsT.txt   -glt_label 1 AvsT
-glt 1 contrasts/contr_HvsL.txt   -glt_label 2 HvsL
-glt 1 contrasts/contr_ATvsHL.txt -glt_label 3 ATvsHL
```

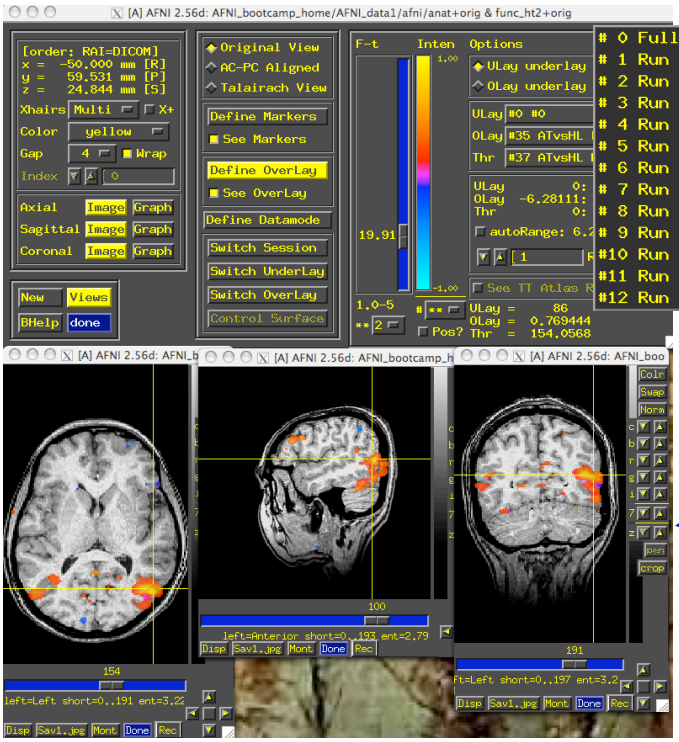
- **GLT**s are General Linear Tests
- **3dDeconvolve** provides tests for each regressor separately, but if you want to test combinations or contrasts of the β weights in each voxel, you need the `-glt` option
- File `contrasts/contr_AvsT.txt` = **0 0 0 0 0 0 0 0 1 -1 0 0** (one line with 12 numbers)

  8 zeros: could also write **8@0**
- Goal is to test a linear combination of the β weights
  - ⋆ In this data, we have 12 β weights: 8 baseline parameters (2 per imaging run), which are first in the β vector, and 4 regressor magnitudes, which are from `-stim_file` options
  - ⋆ This particular test contrasts the Actions and Tool βs
    - tests if $\beta_{Actions} - \beta_{Tool} \neq 0$

Extra Features of 3dDeconvolve - 3

- File `contrasts/contr_HvsL.txt` = **0 0 0 0 0 0 0 0 0 0 1 -1**
  - Goal is to test if $\beta_{HighC} - \beta_{LowC} \neq 0$
- File `contrasts/contr_ATvsHL.txt` = **0 0 0 0 0 0 0 0 1 1 -1 -1**
  - Goal is to test if $(\beta_{Actions} + \beta_{Tool}) - (\beta_{HighC} + \beta_{LowC}) \neq 0$
  - Regions where this statistic is significant will have had different amounts of BOLD signal change in the activity viewing tasks versus the grating viewing tasks
    - This is a way to factor out primary visual cortex
- `-glt_label 3 ATvsHL` option is used to attach a meaningful label to the resulting statistics sub-bricks

Results of decon_ht2 Script



- Menu showing labels from **3dDeconvolve** run
- Images showing results from third contrast: **ATvsHL**
- Play with this yourself to get a feel for it

Statistics from 3dDeconvolve

- An *F*-statistic measures significance of how much a model component reduced the variance of the time series data
- Full *F* measures how much the signal regressors reduced the variance over just the baseline regressors (**sub-brick #0 below**)
- Individual partial-model *F*s measures how much each individual signal regressor reduced data variance over the full model with that regressor excluded (**sub-bricks #19, #22, #25, and #28 below**)

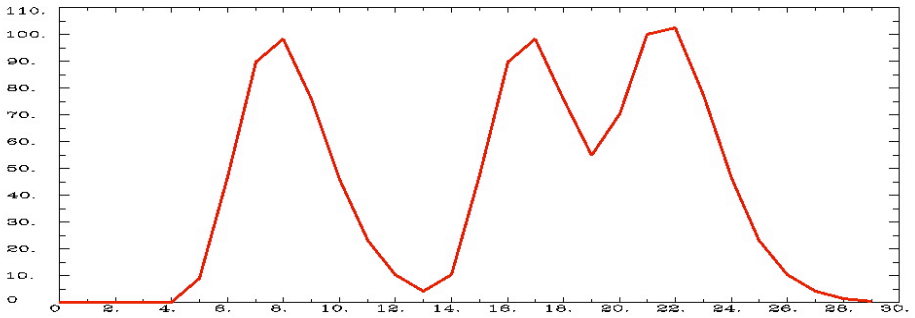
- The **Coef** sub-bricks are the β weights (e.g., **#17, #20, #23, #26**)
- A *t*-statistic sub-brick measure impact of one coefficient

```
# 0 Full F-stat     #13 Run #4 t^0 Coe #26 LowC[0] Coef
# 1 Run #1 t^0 Coe  #14 Run #4 t^0 t-s #27 LowC[0] t-st
# 2 Run #1 t^0 t-s  #15 Run #4 t^1 Coe #28 LowC F-stat
# 3 Run #1 t^1 Coe  #16 Run #4 t^1 t-s #29 AvsT LC[0] coe
# 4 Run #1 t^1 t-s  #17 Actions[0] Coe #30 AvsT LC[0] t-s
# 5 Run #2 t^0 Coe  #18 Actions[0] t-s #31 AvsT F-stat
# 6 Run #2 t^0 t-s  #19 Actions F-stat #32 HvsL LC[0] coe
# 7 Run #2 t^1 Coe  #20 Tool[0] Coef   #33 HvsL LC[0] t-s
# 8 Run #2 t^1 t-s  #21 Tool[0] t-st   #34 HvsL F-stat
# 9 Run #3 t^0 Coe  #22 Tool F-stat    #35 ATvsHL LC[0] c
#10 Run #3 t^0 t-s  #23 HighC[0] Coef  #36 ATvsHL LC[0] t
#11 Run #3 t^1 Coe  #24 HighC[0] t-st  #37 ATvsHL F-stat
#12 Run #3 t^1 t-s  #25 HighC F-stat
```

Alternative Way to Run `waver`

Instead of giving stimulus timing on TR-grid as set of 0s and 1s

- Can give the actual stimulus times (in seconds) using the `-tstim` option
  - ★ `waver -dt 1.0 -GAM -tstim 3 12 17 | 1dplot -stdin`



- If times are in a file, can use `-tstim` `` `cat filename` `` to place them on the command line after `-tstim` option
  - ★ This is most useful for event-related experiments, where the timing of stimuli is usually given explicitly

Note backward single quotes

Alternative Way to Run `3dDeconvolve`

Instead of giving stimulus timing to **waver**

- Can give the actual stimulus times (in seconds) directly to `3dDeconvolve` using the `-stim_times` option (instead of `-stim_file` as before)
- The program will do the equivalent of `waver` inside itself to generate the necessary column(s) in the **R** matrix
- More information in the latter part of this presentation
  - ★ Is coupled with the ideas needed for “deconvolution”
  - ★ Besides input file with stimulus times, must also specify the HRF model to be used with those times
    - o That is, which shape(s) are to be placed down at each stimulus time to model the ideal response

Deconvolution Signal Models

- Simple or Fixed-shape regression (previous):
  - ⋆ We fixed the shape of the HRF — amplitude varies
  - ⋆ Used waver to generate the signal model from the stimulus timing (or could use 3dDeconvolve directly)
  - ⋆ Found the amplitude of the signal model in each voxel — solution to the set linear equations = β weights
- Deconvolution or Variable-shape regression (next):
  - ⋆ We allow the shape of the HRF to vary in each voxel, for each stimulus class
  - ⋆ Appropriate when you don't want to over-constrain the solution by assuming an HRF shape
  - ⋆ ***Caveat***: need to have enough time points during the HRF in order to resolve its shape

Deconvolution: Pros and Cons

+ Letting HRF shape varies allows for subject and regional variability in hemodynamics
+ Can test HRF estimate for different shapes; e.g., are later time points more "active" than earlier?
– Need to estimate more parameters for each stimulus class than a fixed-shape model (e.g., 4-15 *vs*. 1 parameter=amplitude of HRF)
– Which means you need more data to get the same statistical power (assuming that the fixed-shape model you would otherwise use was in fact "correct")
– Freedom to get any shape in HRF results can give weird shapes that are difficult to interpret

Expressing HRF via Regression Unknowns

- The tool for expressing an unknown function as a finite set of numbers that can be fit via linear regression is an **expansion in basis functions**

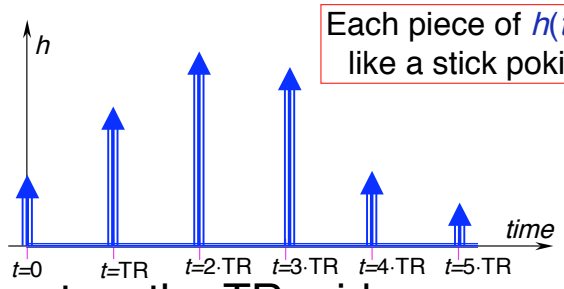
$$h(t) = \beta_0\psi_0(t) + \beta_1\psi_1(t) + \beta_2\psi_2(t) + \cdots = \sum_{q=0}^{q=p} \beta_q\psi_q(t)$$

  - ⋆ The basis functions $\psi_q(t)$ are known, as is the expansion order p
  - ⋆ The unknowns to be found (in each voxel) comprises the set of weights β_q for each $\psi_q(t)$
- Since β weights appear only by multiplying known values, and HRF only appears in final signal model by linear convolution, resulting signal model is still solvable by linear regression

Basis Function: "Sticks"

- The set of basis functions you use determines the range of possible HRFs that you can compute
- "Stick" (or Dirac delta) functions are very flexible
  - ⋆ But they come with a strict limitation
- $\delta(t)$ is 1 at t=0 and is 0 at all other values of t
- $\psi_q(t) = \delta(t - q\cdot\mathrm{TR})$ for q=0,1,2,…,p

  ⇒ $h(0) = \beta_0$
  ⇒ $h(\mathrm{TR}) = \beta_1$
  ⇒ $h(2\cdot\mathrm{TR}) = \beta_2$
  ⇒ $h(3\cdot\mathrm{TR}) = \beta_3$
  ⇒ *et cetera*
  ⇒ $h(t) = 0$ for any t not on the TR grid



Each piece of $h(t)$ looks like a stick poking up

Sticks: Good Points

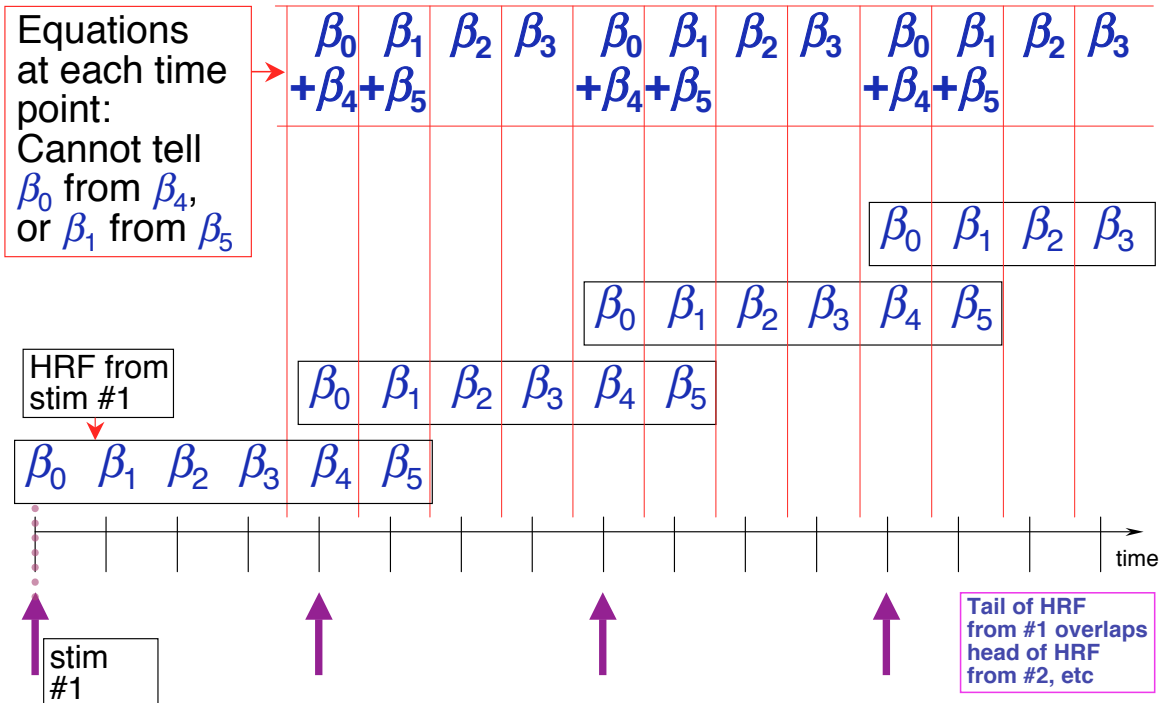
- Can represent arbitrary shapes of the HRF, up and down, with ease
- Meaning of each β_q is completely obvious
  - ⋆ Value of HRF at time lag $q \cdot \mathrm{TR}$ after activation
- **3dDeconvolve** is set up to deal with stick functions for representing HRF, so using them is very easy
  - What is called p here is given by command line option `-stim_maxlag` in the program
  - When choosing p, rule is to estimate longest duration of neural activation after stimulus onset, then add 10-12 seconds to allow for slowness of hemodynamic response

Sticks and TR-locked Stimuli

- **$h(t) = 0$ for any t not on the TR grid**
- This limitation means that, using stick functions as our basis set, we can only model stimuli that are "locked" to the TR grid
  - ⋆ That is, stimuli/activations don't occur at fully general times, but only occur at integer multiples of TR
- For example, suppose an activation is at $t = 1.7 \cdot \mathrm{TR}$
  - ⋆ We need to model the response at later times, such as $2 \cdot \mathrm{TR}$, $3 \cdot \mathrm{TR}$, etc., so need to model $h(t)$ at times such as $t = (2 - 1.7) \cdot \mathrm{TR} = 0.3 \cdot \mathrm{TR}$, $t = 1.3 \cdot \mathrm{TR}$, etc., after the stimulus
  - But the stick function model doesn't allow for such intermediate times
    - ***or***, can allow Δt for sticks to be a fraction of TR for data
    - e.g., $\Delta t = \mathrm{TR}/2$, which implies twice as many β_q parameters to cover the same time interval (time interval needed is set by hemodynamics)
    - then would allow stimuli that occur on TR-grid or halfway in-between

Deconvolution and Collinearity

- Regular stimulus timing can lead to collinearity!



3dDeconvolve with Stick Functions

- Instead of inputting a signal model time series (e.g., created with **waver** and stimulus timing), you input the stimulus timing directly
  - ★ Format: a text file with 0s and 1s, 0 at TR-grid times with no stimulus, 1 at time with stimulus
- Must specify the maximum lag (in units of TR) that we expect HRF to last after each stimulus
  - ★ This requires you to make a judgment about the activation — brief or long?
- **3dDeconvolve** returns estimated values for each β_q, for each stimulus class
  - ★ Usually then use a GLT to test the HRF (or pieces of it) for significance

Extra Features of 3dDeconvolve - 4

- **`-stim_maxlag k p`** = option to set the maximum lag to **`p`** for stimulus timing file #**`k`** for **`k=0,1,2,...`**
  - ★ Stimulus timing file input using command line option **`-stim_file k filename`** as before
  - ★ Can also use **`-stim_minlag k m`** option to set the minimum lag if you want a value **`m`** different from **`0`**
  - ★ In which case there are **`p-m+1`** parameters in this HRF
- **`-stim_nptr k r`** = option to specify that there are **`r`** stimulus subintervals per TR, rather than just 1
  - ★ This feature can be used to get a finer grained HRF, at the cost of adding more parameters that need to be estimated
  - • Need to make sure that the input stimulus timing file (from **`-stim_file`**) has **`r`** entries per TR
  - • TR for **`-stim_file`** and for output HRF is data TR ÷ **`r`**

Script for Deconvolution - The Data

- **`cd AFNI_data2`**
  - ★ data is in **`ED/`** subdirectory (10 runs of 136 images each; TR=2 s)
  - ★ script in file **`@s1.analyze_ht05`** (in **`AFNI_data2`** directory)
    - o stimuli timing and GLT contrast files in **`misc_files/`**
  - ★ start script ***now*** by typing **`source @s1.analyze_ht05`**
    - o will discuss details of script while it runs (20+ min?)
- Event-related study from Mike Beauchamp ↔
  - • Formerly LBC/NIMH
  - • Now UT Houston
  - ★ 10 runs with four classes of stimuli (short videos)
    - o Tools moving (e.g., a hammer pounding) - **TM**
    - o People moving (e.g., jumping jacks) - **HM**
    - o Points outlining tools moving (no objects, just points) - **TP**
    - o Points outlining people moving - **HP**
  - ★ Goal is to find if there is an area that distinguishes natural motions (HM and HP) from simpler rigid motions (TM and TP)

Script for Deconvolution - Outline

- Examine each imaging run for outliers: **3dToutcount**
- Time shift each run's slices to a common origin: **3dTshift**
- Registration of each imaging run: **3dvolreg**
- Smooth each volume in space (136 sub-bricks per run): **3dmerge**
- Create a brain mask: **3dAutomask** and **3dcalc**
- Rescale each voxel time series in each imaging run so that its average through time is 100: **3dTstat** and **3dcalc**
  - ⋆ If baseline is 100, then a β_q of 5 (say) indicates a 5% signal change in that voxel at time laq #q after stimulus
- Catenate all imaging runs together into one big dataset (1360 time points): **3dTcat**
- Compute HRFs and statistics: **3dDeconvolve**
  - ⋆ Each HRF will have 15 time points (lags from 0 to 14) with TR=1.0 s, since input data has TR=2.0 s and we use **-stim_nptr k r** option with **r=2**
- Average together all points of each separate HRF to get average % change in each voxel over 14 s interval: **3dTstat**

Script for Deconvolution - 1

```
#!/bin/tcsh

if ( $#argv > 0 ) then
    set subjects = ( $argv )
else
    set subjects = ED
endif
```

This script is designed to run analyses on a lot of subjects at once. We will only analyze the ED data here. The other subjects will be included in the Group Analysis presentation.

```
#=========================================================================
# Above command will run script for all our subjects - ED, EE, EF - one after
# the other if, when we execute the script, we type: ./@s1.analyze_ht05 ED EE EF.
# If we type ./@s1.analyze_ht05 or tcsh @s1.analyze_ht05, the script runs only
# for subject ED.  The user will then have to go back and edit the script so
# that 'set subjects' = EE and then EF, and then run the script for each subj.
#=========================================================================

foreach subj ($subjects)
```

Loop over all subjects (next 2 slides)

```
    cd $subj
```

First step is to change to the directory that has this subject's data

Script for Deconvolution - 2

```
#=======================================================================
# time shift, volume register and spatially blur our datasets,
# and remove the first two time points from each run
#=======================================================================

set runs = ( `count -digits 2 1 10` )
foreach run ( $runs )


   set dset = ${subj}_r${run}+orig.HEAD


   3dToutcount -automask ${dset}        \
               > toutcount_r$run.1D


   3dTshift -tzero 0 -heptic            \
            -prefix ${subj}_r${run}_ts \
             ${dset}
```

Loop over imaging runs 1..10
(loop continues on next slide)

Shorthand for dataset

Outlier check:
By itself, `3dToutcount` doesn't change data!
To plot "outlier-ness":
`1dplot toutc_r1.1D`

Interpolate each voxel's time series to start at the time of slice #0

Script for Deconvolution - 3

```
   3dvolreg -verbose                                \
            -base ${subj}_r01_ts+orig'[2]'    \
            -prefix ${subj}_r${run}_vr        \
            -1Dfile dfile.r$run.1D            \
            ${subj}_r${run}_ts+orig'[2..137]'


   3dmerge -1blur_fwhm 4                        \
           -doall                               \
           -prefix ${subj}_r${run}_vr_bl \
           ${subj}_r${run}_vr+orig


   3dAutomask -dilate 1                        \
              -prefix mask_r${run}       \
              ${subj}_r${run}_vr_bl+orig
```

Image registration of each run to its #2 sub-brick

Lightly blur each 3D volume in each dataset to reduce noise and increase functional overlap among runs and among subjects

Make an "inside-the-brain" mask for this dataset

```
end
```

End of loop over imaging runs.
At this point, dataset `${subj}_r${run}_vr_bl` contains the data for subject `${subj}` and imaging run `${run}`, which has been time-shifted, realigned, and blurred; also, a brain-only mask has been made

Script for Deconvolution - 4

```
#=======================================================================
# create a union mask from those of the individual runs
#=======================================================================
3dcalc -a mask_r01+orig -b mask_r02+orig -c mask_r03+orig \
       -d mask_r04+orig -e mask_r05+orig -f mask_r06+orig \
       -g mask_r07+orig -h mask_r08+orig -i mask_r09+orig \
       -j mask_r10+orig                                   \
       -expr 'or(a+b+c+d+e+f+g+h+i+j)'                    \
       -prefix full_mask
```

This mask dataset will be 1 inside the largest contiguous high intensity EPI region, and 0 outside that region — this makes a brain mask

3dcalc program = voxel-wise "calculator" for datasets.
Input is 10 individual run dataset masks (1 in brain, 0 outside).
Output is mask which is

- 1 wherever *any* individual mask is 1,
- 0 wherever *all* individual masks are 0

Script for Deconvolution - 5

```
#=========================================================================
# - re-scale each run's mean to 100
# - use full_mask to zero out non-brain voxels
#
# If the mean is 100, and the result of 3dcalc at a voxel is 106 (at
# some time point), then one can say that voxel shows a 6% increase in
# signal activity, relative to the mean.
#=========================================================================

foreach run ( $runs )

  3dTstat -prefix mean_r${run}                \
          ${subj}_r${run}_vr_bl+orig

  3dcalc -a ${subj}_r${run}_vr_bl+orig        \
         -b mean_r${run}+orig                 \
         -c full_mask+orig                    \
         -expr "(a/b * 100) * c"              \
         -prefix scaled_r${run}

  rm -f mean_r${run}+orig*

end
```

Mean of the runth dataset, through time: run=1..10

- Divide each voxel value ('**a**') by its temporal mean ('**b**') and multiply by 100
- Result will have temporal mean of 100
- Voxels not in the mask will be set to 0 (by '**c**')

Script for Deconvolution - 6

```
3dTcat -prefix ${subj}_all_runs \
       scaled_r??+orig.HEAD
```

"Gluing" the runs together, since **3dDeconvolve** only operates on one input dataset at a time

```
cat dfile.r??.1D > dfile.all.1D
```

Also "glue" together the movement parameters output from **3dvolreg**

```
#=========================================================
# move unloved run data into separate directories
#=========================================================

mkdir runs_orig runs_temp

mv ${subj}_r*_vr* ${subj}_r*_ts* scaled* \
   dfile.r??.1D toutcount* runs_temp

mv ${subj}_r* runs_orig
```

Gets this stuff out of the way so that we don't see it when we run AFNI later

Script for Deconvolution - 7

```
3dDeconvolve -polort 2                                          \
  -input ${subj}_all_runs+orig -num_stimts 10                   \
  -concat ../misc_files/runs.1D                                 \
  -stim_file 1  ../misc_files/all_stims.1D'[0]'                 \
     -stim_label 1 ToolMovie                                    \
     -stim_minlag 1 0 -stim_maxlag 1 14 -stim_nptr 1 2          \
  -stim_file 2  ../misc_files/all_stims.1D'[1]'                 \
     -stim_label 2 HumanMovie                                   \
     -stim_minlag 2 0 -stim_maxlag 2 14 -stim_nptr 2 2          \
  -stim_file 3  ../misc_files/all_stims.1D'[2]'                 \
     -stim_label 3 ToolPoint                                    \
     -stim_minlag 3 0 -stim_maxlag 3 14 -stim_nptr 3 2          \
  -stim_file 4  ../misc_files/all_stims.1D'[3]'                 \
     -stim_label 4 HumanPoint                                   \
     -stim_minlag 4 0 -stim_maxlag 4 14 -stim_nptr 4 2          \
```

Input dataset

0-1 stim file #1

0-1 stim file #2

0-1 stim file #3

0-1 stim file #4

- 4 time series models: one for each the 4 different classes of events
- All stimuli time series in 1 file with 4 columns: **../misc_files/all_stims.1D**
  - Selectors like '**[2]**' pick out a particular column
  - Each stimulus input and HRF output is sampled at TR/2 = 1.0 s
    - Due to the use of **-stim_nptr k 2** for each **k**
  - Lag from 0 to 14 s is about right for HRF to a brief stimulus
- **-stim_label** option: names used in AFNI and below in **-gltsym** options

Script for Deconvolution - 8

```
-stim_file 5  dfile.all.1D'[0]' -stim_base 5  \
-stim_file 6  dfile.all.1D'[1]' -stim_base 6  \
-stim_file 7  dfile.all.1D'[2]' -stim_base 7  \
-stim_file 8  dfile.all.1D'[3]' -stim_base 8  \
-stim_file 9  dfile.all.1D'[4]' -stim_base 9  \
-stim_file 10 dfile.all.1D'[5]' -stim_base 10 \

-iresp 1 TMirf -iresp 2 HMirf                 \
-iresp 3 TPirf -iresp 4 HPirf                 \
-full_first -fout -tout -nobout -xjpeg Xmat   \
-bucket ${subj}_func                          \
```

Movement regressors-of-no-interest: output from `3dvolreg`

- Output HRF (`-iresp`) 3D+time dataset for each stimulus class
  - Each of these 4 datasets will have TR=1.0 s and have 15 time points (β weights for lags 0..14)
  - Can plot these HRF datasets atop each other using `Dataset#N` plugin
  - Useful for visual inspection of regions that GLTs tell you have different responses for different classes of stimuli
- `-nobout` = don't output statistics of baseline parameters
- `-bucket` = save statistics into dataset with this prefix
- `-xjpeg` = save an image of the **R** matrix into file `Xmat.jpg`

Script for Deconvolution - 9

```
-gltsym ../misc_files/contrast1.1D -glt_label 1 FullF     \
-gltsym ../misc_files/contrast2.1D -glt_label 2 HvsT      \
-gltsym ../misc_files/contrast3.1D -glt_label 3 MvsP      \
-gltsym ../misc_files/contrast4.1D -glt_label 4 HMvsHP    \
-gltsym ../misc_files/contrast5.1D -glt_label 5 TMvsTP    \
-gltsym ../misc_files/contrast6.1D -glt_label 6 HPvsTP    \
-gltsym ../misc_files/contrast7.1D -glt_label 7 HMvsTM
```

- Run many GLTs to contrast various pairs and quads of cases
- New feature: `-gltsym` = specify β weights to contrast using `-stim_label` names given earlier on the command "line"
  - Simpler than counting 0s and ±1s to fill out GLT matrix numerically
- Example: file `contrast2.1D` is the single line below:

  `-ToolMovie +HumanMovie -ToolPoint +HumanPoint`

  which means to put "-1" in the matrix for all 15 lags for stimuli #1 and #3 and "+1" in the matrix for all 15 lags for stimuli #2 and #4
  - This is the "Human vs Tools" contrast (labeled `HvsT` via `-glt_label`)
  - Sum of the 30 "Tool" β weights subtracted from Sum of the 30 "Human" β weights
  - Testing: % signal change for Human stimuli different than Tool stimuli?

Script for Deconvolution - 10

```
  3dbucket -prefix ${subj}_func_slim -fbuc  \
           ${subj}_func+orig'[0,125..151]'

  foreach cond (TM HM TP HP)

    3dTstat -prefix ${subj}_${cond}_irf_mean \
            ${cond}irf+orig

    adwarp -apar ${subj}spgr+tlrc -dxyz 3 \
           -dpar ${subj}_${cond}_irf_mean+orig
  end

  cd ..
end

#==========================================================
# End of script!
# Take ${subj}_${cond}_irf_mean+tlrc datasets into 3dANOVA3
#==========================================================
```

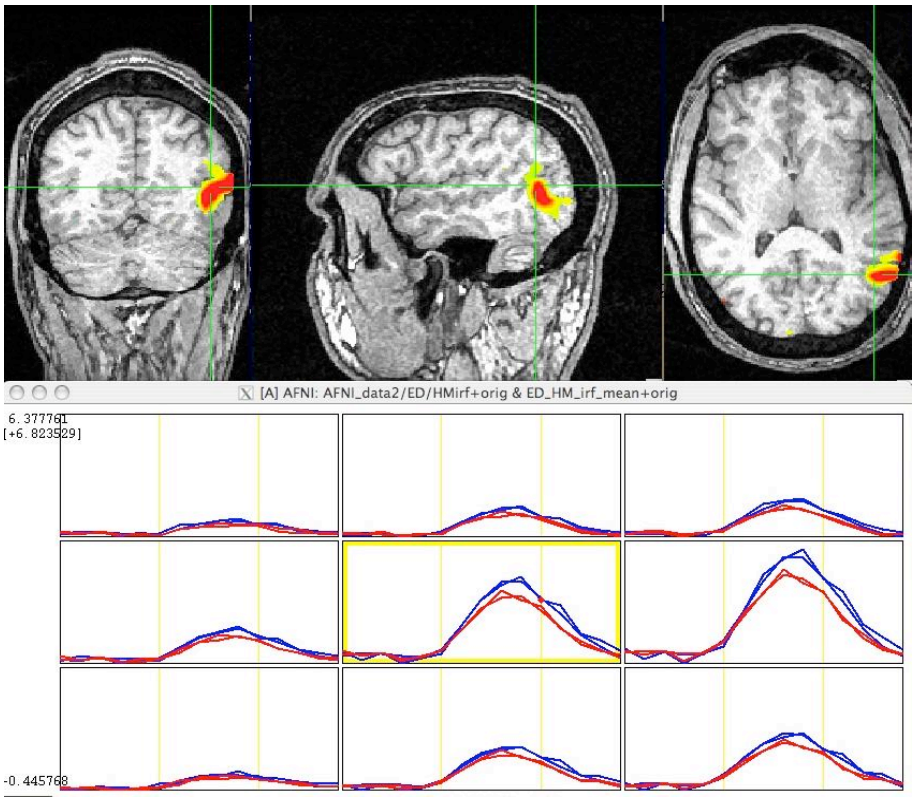
Extract a subset of interesting statistics sub-bricks into a "slimmed-down" functional dataset

Compute HRF means across all lags 0..14 for each of the 4 stimuli types

Transform this individual's mean % signal results into Talairach coordinates for group analyses

End of loop over subjects; go back to upper directory whence we started

Results: Humans vs. Tools



- Color overlay is **HvsT** contrast
- **Blue** (upper) curves: Human HRFs
- **Red** (lower) curves: Tool HRFs

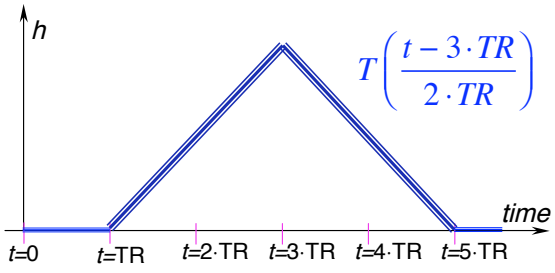
Yet More Fun 3dDeconvolve Options

- **`-mask`** = used to turn off processing for some voxels
  - ★ speed up the program by not processing non-brain voxels
- **`-input1D`** = used to process a single time series, rather than a dataset full of time series
  - ★ test out a stimulus timing sequence
  - ★ **`-nodata`** option can be used to check for collinearity
- **`-censor`** = used to turn off processing for some time points
  - ★ for time points that are "bad" (e.g., too much movement)
- **`-sresp`** = output standard deviation of HRF estimates
  - ★ can plot error bands around HRF in AFNI graph viewer
- **`-errts`** = output residuals (i.e., difference between fitted model and data)
  - ★ for statistical analysis of time series noise
- **`-jobs`** ***N*** = run with multiple CPUS — ***N*** of them
  - ★ extra speed, if you have a dual-CPU system (or more)!

3dDeconvolve with Free Timing

- The fixed-TR stick function approach doesn't work well with arbitrary timing of stimuli
  - ★ When subject actions/reactions are self-initiated, timing of activations cannot be controlled
- If you want to do deconvolution (vs. fixed-shape analysis), then must adopt a different basis function expansion approach
  - ★ One that has a finite number of parameters but also allows for calculation of ***h(t)*** at any arbitrary point in time
- Simplest set of such functions are closely related to stick functions: **tent functions**

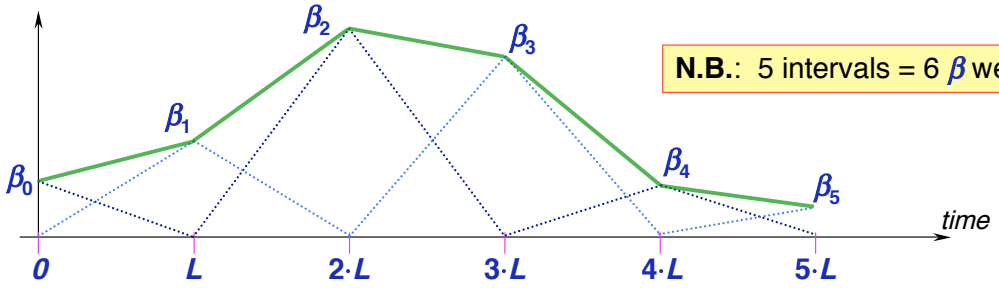
$$T(x) = \begin{cases} 1-|x| & \text{for } -1 < x < 1 \\ 0 & \text{for } |x| > 1 \end{cases}$$



Tent Functions = Linear Interpolation

- Expansion in a set of spaced-apart tent functions is the same as linear interpolation

$$\beta_0 \cdot T\left(\frac{t}{L}\right) + \beta_1 \cdot T\left(\frac{t-L}{L}\right) + \beta_2 \cdot T\left(\frac{t-2\cdot L}{L}\right) + \beta_3 \cdot T\left(\frac{t-3\cdot L}{L}\right) + \cdots$$



N.B.: 5 intervals = 6 β weights

- Tent function parameters are also easily interpreted as function values (e.g., β_2 = response at time $t = 2 \cdot L$ after stim)
- User must decide on relationship of tent function grid spacing L and time grid spacing TR (usually would choose $L \geq$ TR)
- Fancy name for tent functions: piecewise linear B-splines

Tent Functions: Average Signal Change

- For input to group analysis, usually want to compute average signal change
  - ⋆ Over entire duration of HRF (usual)
  - ⋆ Over a sub-interval of the HRF duration (sometimes)
- In previous slide, with 6 β weights, average signal change is

$$^1/_2\,\beta_0 + \beta_1 + \beta_2 + \beta_3 + \beta_4 + {}^1/_2\,\beta_5$$

- First and last β weights are scaled by half since they only affect half as much of the duration
- In practice, may want to use $0 \cdot \beta_0$ since immediate post-stimulus response is not hemodynamically correct
- β weights are output into the "bucket" dataset produced by `3dDeconvolve`
- Can then be combined into a single number using `3dcalc`

3dDeconvolve -stim_times

- Direct input of stimulus timing, plus a response model
- Specifies stimuli, instead of using `-stim_file`
- `-stim_times k tname rtype`
  - ★ `k` = stimulus index (from 1 to `-num_stimts` value)
- `tname` = name of `.1D` file containing stimulus times (seconds)
  - ★ ***N.B.***: TR stored in dataset header must be correct!
- `rtype` = name of response model to use for each stimulus time read from `tname` file
  - ★ `GAM` = gamma variate function from `waver` (fixed-shaped analysis)
  - ★ `TENT(b,c,n)` = tent function deconvolution, ranging from time `s+b` to `s+c` after each stimulus time `s`, with `n` basis functions (divided evenly over `c-b` seconds, into `n-1` intervals)
  - ★ several other `rtype` options available (experimental)
- Can mix `-stim_file` and `-stim_times` as needed
  - ★ e.g., movement parameter regressors at each TR

Two Possible Formats of Timing File

- A single column of numbers

```
 4.7
 9.6
11.8
19.4
```

  - ★ One stimulus time per row
  - ★ Times are relative to first image in dataset being at ***t=0***
  - ★ May not be simplest to use if multiple runs are catenated
- One row for each run within a catenated dataset
  - ★ Each time in *j*th row is relative to start of run #*j* being ***t=0***
  - ★ If some run has NO stimuli in the given class, just put a single "`*`" in that row as a filler
    - Different numbers of stim per run are OK
    - At least one row must have more than 1 time (so that this type of timing file can be told from the other)

```
4.7 9.6 11.8 19.4
*
8.3 10.6
```

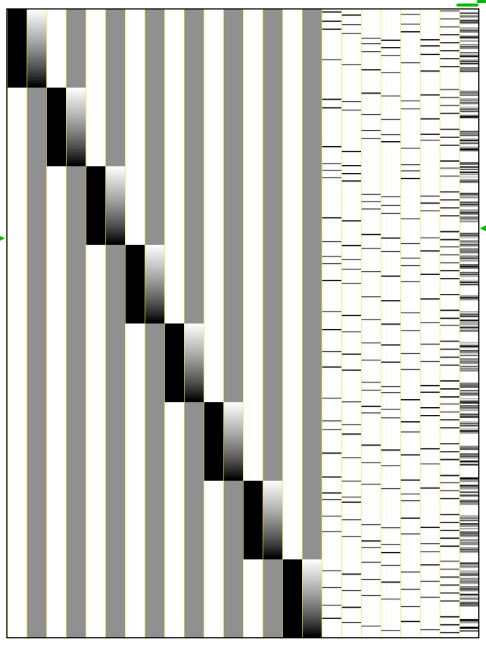
- Two methods are available because of users' diverse needs
  - ★ **N.B.**: if you chop first few images off the start of each run, the inputs to `-stim_times` must be adjusted accordingly

Other Recent-ish Upgrades

- See http://afni.nimh.nih.gov/doc/misc/3dDeconvolveSummer2004/
- Equation solver: Gaussian elimination to compute **R** matrix pseudo-inverse was replaced by SVD (like principal components)
  - ★ Advantage: smaller sensitivity to computational errors
  - ★ "Condition number" and "inverse error" values are printed at program startup, as measures of accuracy of pseudo-inverse
  - ★ Condition number < 1000 is good
  - ★ Inverse error < 1.0e-10 is good
- `3dDeconvolve_f` program can be used to compute in single precision (7 decimal places) rather than double precision (16)
  - ★ For better speed, but with lower numerical accuracy
  - ★ Best to do at least one run **both** ways to check if results differ significantly (SVD solver *should* be safe)

Recent Upgrades - 2

- New `-xjpeg xxx.jpg` option will save a JPEG image file of the columns of the **R** matrix into file `xxx.jpg` (and an image of the pseudo-inverse of **R** into file `xxx_psinv.jpg`)



Constant and linear baselines for each run (`-polort 1`)

Simple regression functions created by `waver` and input by `-stim_file`

Why 'x' instead of 'R'? Because SPM calls this the 'X' matrix, not the 'R' matrix.

Recent Upgrades - 3

- Matrix inputs for `-glt` option can now indicate lots of zero entries using a notation like `30@0 1 -1 0 0` to indicate that 30 zeros precede the rest of the input line
  - ⋆ Example: 10 imaging runs and `-polort 2` for baseline
  - ⋆ Can put comments into matrix and .1D files, using lines that start with '`#`' or '`//`'
  - ⋆ Can use '`\`' at end of line to specify continuation
- Matrix input for GLTs can also be expressed symbolically, using the names given with the `-stim_label` options:

```
-stim_label 1 Ear -stim_maxlag 1 4
-stim_label 2 Wax -stim_maxlag 2 4
```

  - ⋆ Old style GLT might be

Sum of Ear – Sum of Wax (lags 2..4)

```
{zeros for baseline} 0 0 1 1 1 0 0 -1 -1 -1
```

  - ⋆ New style (via `-gltsym` option) is

```
Ear[2..4] -Wax[2..4]
```


Recent Upgrades - 4

- New `-xsave` option saves the **R** matrix (and other info) into a file that can be used later with the `-xrestore` option to calculate some extra GLTs, without re-doing the entire analysis (goal: save some time by not recomputing)
- `-input` option now allows multiple 3D+time datasets to be specified to automatically catenate individual runs into one file 'on the fly'
  - ⋆ Avoids having to use program `3dTcat`
  - ⋆ User must still supply full-length `.1D` files for the various input time series (e.g., `-stim_file`, `-stim_times`)
  - ⋆ `-concat` option will be ignored if this option is used
    - o Break points between runs will be taken as the break points between the various `-input` datasets
- `-polort` option now uses Legendre polynomials instead of simple 1, t, t^2, t^3, … basis functions (more numerical accuracy)

Recent Upgrades - 5

- **3dDeconvolve** now checks for duplicate **-stim_file** names and for duplicate matrix columns, and prints warnings
  - ⋆ These are not fatal errors
    - o If the same regressor is given twice, each copy will only get half the amplitude (the “beta weight”) in the solution
- All-zero regressors are now allowed
  - ⋆ Will get zero weight in the solution
    - o A warning message will be printed to the terminal
  - ⋆ Example: task where subject makes a choice for each stimulus (e.g., male or female face?)
    - o You want to analyze correct and incorrect trials a separate cases
    - o What if a subject makes no mistakes? Hmmm…

Recent Upgrades - 6

- Recall: **-iresp** option outputs the HRF model for one stimulus
  - ⋆ When used with **-stim_times**, values are usually output using the dataset TR time spacing
  - ⋆ Can changes to a different grid via new **-TR_times dt** option, which sets the output grid spacing for **-iresp** to **dt** for HRF models computed via **-stim_times**
    - o Is useful for producing nice smooth pictures of HRF
    - o Also works with **-sresp** option (= std.dev. of HRF)
- **Difficulty**: using GLTs with results from **-stim_times**
  - ⋆ GLTs operate on regression coefficients
  - ⋆ For advanced (experimental) **rtype** models, regression coefficients don’t correspond directly to HRF amplitudes
    - o Exceptions: **GAM**, **TENT**, **BLOCK**

Upgrades – *Planned* or *Dreamed of*

- Automatic baseline normalization of input time series
- Automatic mask generation (à la `3dAutomask` program)
- Spatial blur (à la `3dmerge -1blur)`
- Time shift input before analysis (à la `3dTshift` program)
- Negative lags for `-stim_file` method of deconvolution
  - ⋆ for pre-stimulus cognition/anticipation
  - ⋆ `-stim_times` already allows pre-stimulus response
- 'Area under curve' addition to `-gltsym` to allow testing of pieces of HRF models from `-stim_times`
- Slice- and/or voxel-dependent regressors
  - ⋆ For physiological noise cancellation, etc.
- Floating point output format
  - ⋆ Currently is shorts + scale factor

Advanced Topics in Regression

- Can have activations with multiple phases that are not always in the same time relationship to each other; e.g.:
  a) subject gets cue #1
  b) variable waiting time ("hold")
  c) subject gets cue #2, emits response
     - ➥ which depends on both cue #1 and #2

  } timing of events is known

  - ⋆ Cannot treat this as one event with one HRF, since the different waiting times will result in different overlaps in separate responses from cue #1 and cue #2
  - ⋆ Solution is multiple HRFs: separate HRF (fixed shape or deconvolution) for cue #1 times and for cue #2 times
    - o Must have significant variability in inter-cue waiting times, or will get a nearly-collinear model
      - ➥ impossible to tell tail end of HRF #1 from the start of HRF #2, if always locked together in same temporal relationship
    - o How much variability is "significant"? Good question.

Even More Complicated Case

- Solving a visually presented puzzle:
  a) subject sees puzzle
  b) subject cogitates a while
  c) subject responds with solution

  } timing of events is measured
- The problem is that we expect some voxels to be significant in phase (b) as well as phases (a) and/or (c)
- Variable length of phase (b) means that shape for its response varies between trials
  - ★ Which is contrary to the whole idea of averaging trials together to get decent statistics (which is basically what linear regression amounts to, in a fancy sort of way)
- Could assume response ***amplitude*** in phase (b) is constant across trials, and response ***duration*** varies directly with time between phases (a) and (c)
  - ★ Need three HRFs; phase (b)'s is a little tricky to generate using waver, but it could be done

Noise Issues

- "Noise" in FMRI is caused by several factors, not completely characterized
  - ★ MR thermal noise (well understood, unremovable)
  - ★ Cardiac and respiratory cycles (partly understood)
    - o In principle, could measure these sources of noise separately and then try to regress them out
      - ➥ RETROICOR program underway (R Birn & M Smith of FIM/NIMH)
  - ★ Scanner fluctuations (e.g., thermal drift of hardware)
  - ★ Small subject head movements (10-100 mm)
  - ★ Very low frequency fluctuations (periods longer than 100 s)
- Data analysis should try to remove what can be removed and allow for the statistical effects of what can't be removed
  - ★ "Serial correlation" in the noise time series affects the *t*- and *F*-statistics calculated by `3dDeconvolve`
  - ★ At present, nothing is done to correct for this effect (by us)

Nonlinear Regression

- Linear models aren't everything
  - ⋆ e.g., could try to fit HRF of the form $h(t) = a \cdot t^b \cdot e^{-t/c}$
  - ⋆ Unknowns ***b*** and ***c*** appear nonlinearly in this formula
- Program **`3dNLfim`** can do nonlinear regression (including nonlinear deconvolution)
  - ⋆ User must provide a C function that computes the model time series, given a set of parameters (e.g., ***a***, ***b***, ***c***)
  - ⋆ Program then drives this C function repeatedly, searching for the set of parameters that best fit each voxel
  - ⋆ Has been used to fit pharmacological wash-in/wash-out models (difference of two exponentials) to FMRI data acquired during pharmacological challenges
    - o e.g., injection of nicotine, cocaine, ethanol, etc.
    - o these are tricky experiments, at best